THE COAGULATION TIME OF THE BLOOD IN MAN.

A PHYSIOLOGICAL STUDY.

THESIS for the DEGREE of M.D., (Edin.Univ.)

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PREFACE.

Since 1897 when Vierordt published his method of estimating the coagulation time of the blood in man, eleven others have appeared.

After working for some time with two of the more recent of them, I found that my results were discordant and unreliable.

I proceeded to a practical study of all the methods with the result that I came to the conclusion that there were certain essential conditions which required to be observed if accurate comparative results were to be achieved. None of the methods complied with all these conditions and in particular they all failed to exclude the disturbing influence of slight temperature variations, and it is mainly on this account that the conclusions arrived at by different observers have been so contradictory.

I have succeeded in devising a method in which the temperature can be kept constant and in which the various other fallacies have to a great extent been avoided. By means of it I have been able to demonstrate the effect of different temperatures on the coagulation time and to show that the diurnal/

diurnal variations in the time which have been differently described, in reality do not occur at all.

After showing that the administration of Calcium and Citric Acid produce alterations in the amount of ionisable Calcium in the blood, I have shown that nevertheless they have no appreciable effect on the coagulation time.

S E C T I O N I.

SECTION I.

I. THE ESSENTIALS FOR A METHOD OF ESTIMATING THE COAGULATION TIME OF THE BLOOD IN MAN.

1. The obtaining of the blood under the same conditions in each experiment.

2. The maintenance of the blood at a constant temperature in all comparative observations.

3. The contact of the blood with exactly the same amount and kind of foreign body in each observation.

4. A clear and definite end-point which shall be the result of the same degree of coagulation in each experiment.

I have been led to the conclusion, that any accurate method must comply with the above conditions, by a comparative study of the methods described in Section/

Section II. This section would, therefore, have more logically followed a description of these methods, and of the results obtained by their use, but I have put it first because on it, I wish to base my criticism of them.

Whenever it has been possible I have shown by the method described in Section III* the variations in the coagulation time which follow on the neglect of any of these conditions.

I believe that I have been able to show that the regular, daily variations which some have described (See Section IV) do not exist, and therefore no attempt has been made to make these observations at the same hours every day.

I think it is almost certain, that the differences between the times, are due not to real variations in the coagulation time, but to experimental error. The amount of error with my method, though considerable, is less than that which is obtained by the use of any other.

* My method.

I. THE OBTAINING OF THE BLOOD UNDER THE SAME CONDITIONS IN EACH EXPERIMENT.

1. The extent to which the wound influences the coagulation time.

2. Effect of variations in the size of the wound.

3. Effect of congestion of the part into which the wound is made.

4. Effect of pressure near the wound.

5. Effect of the rate of flow of blood from the wound.

6. The parts of the body from which the blood is taken.

7. The point in a haemorrhage at which a specimen of the blood should be taken.

8. The condition of the skin surface surrounding the wound.

9. Conclusions.

I. THE EXTENT TO WHICH THE WOUND INFLUENCES THE COAGULATION TIME.

The influence on the coagulation time of a small puncture wound such as is made to obtain a drop of blood can be estimated by excluding all other factors leading to coagulation. This may be attained by puncturing the finger while it is immersed under oil. Oil is neutral as regards the blood, it does not act as a foreign body and neither retards nor hastens coagulation. (1).

Blood so obtained and kept surrounded on all sides by oil coagulates only because of changes which have taken place in it, as a consequence of its passage over the tissues exposed in the wound. Under the above conditions I found that coagulation did not take place for from 70 to 80 minutes, whereas drops of blood drawn in the ordinary way and exposed to contact with air and clean glass coagulated in less than 5 minutes. The details of these experiments are given later, when the influence of different foreign bodies on the coagulation time is discussed. They show/

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(1) Ann. Instit. Pasteur 190/

show that the actual effect of the wound itself is relatively unimportant; it is the environment of the blood after it has issued from the wound which is most powerful in bringing about coagulation.

Nevertheless it has been stated that variations in the coagulation time ~~as~~ occur in connection with differences in the depth of the wound, and also according as the part in which the wound is made, is or is not congested. Pressure near the wound is also supposed to have an effect.

2. EFFECT OF VARIATIONS IN THE SIZE OF THE WOUND.

Pratt[1] found that blood from deep wounds took longer to coagulate than blood from small superficial punctures, and he gives as an example a coagulation time of 7 min. when the cut was deep and of 2 min. when it was superficial.

I attempted therefore to determine whether such variations in the depth of the wound as are likely to occur in pricking the fingers to obtain an immediate drop of blood have any appreciable effect on/

(1) Journal. Med. Research 1903 V 120.

on the coagulation time. A Jenner's vaccinostyle was embedded in a stick of vulcanite. A brass ring about half an inch in diameter was then screwed on to it so that only the point of the lancet came beyond it. By screwing this ring up or down the amount of projecting point could be increased or diminished. The fingers were then pricked alternately first with a small, and with a large amount of the triangular point projecting, so that both the depth and the breadth of the wounds varied considerably.

D.McR.

SMALL WOUND.	LARGE WOUND.
min.sec.	min.sec.
7 55	7 0
7 15	8 10
8 0	7 10
8 27	8 15
8 52	9 5
9 0	9 0
7 50	7 40
7 18	8 5

The average coagulation time of the blood from the small wounds is 8 min 4 sec and the average from/

from the large is 8 min 8sec, so that there is practically no difference.

3.EFFECT OF CONGESTION OF THE PART INTO WHICH THE WOUND IS MADE.

Several observers have stated that congestion leads to a diminution of the time, the explanation being that a greater quantity of coagulation accelerating substances from the tissues are added to the issuing blood in that condition.

Vierordt (1) is the only one who has given any experimental proof of this. The following are the results he obtained.

UNCONGESTED	CONGESTED
min.sec.	min.sec.
12 5	11 25
12 0	7 5
13 0	10 5
9 75	8 25
9 75	7 5
11 5	7 0
10 25	6 25

I/

(1) Archiv d. Heilk. Leipz.1878 XIX 193

I have not been able to confirm these results. A condition of marked congestion of one arm was produced by the application of a Bier's bandage above the elbow. The coagulation time of the blood, from the fingers of that hand, was compared with the time in the fingers of the other hand.

T.A.

UNCONGESTED	CONGESTED.
min.sec.	min.sec.
7 40	8 5
7 23	8 5
7 5	7 13
7 0	7 7
7 0	7 10

A.M.

8. 30	8 0
7 30	8 15
9 25	8 45
8 0	7 30

M.M.

7 40	8 50
8 50	8 55
8 17	8 35

THE/

The average coagulation time in the uncongested fingers is 7 min 51 sec., and in the congested 8 min 2 sec. No conclusion can be drawn from such a slight difference as 11 seconds.

The warning given by Hingston Fox[1] and others against producing temporary congestion to aid the flow of blood from the wound may, therefore, be disregarded as it has no appreciable effect, one way or the other.

4. EFFECT OF PRESSURE NEAR THE WOUND.

This is supposed to act in the same way as congestion, namely by expressing fluids from the tissues, into the blood, as it flows from the wound. Milian[2] found that the last drops squeezed from a wound at the end of a long capillary haemorrhage, coagulated much more quickly than the blood taken at any other period. He attributed this to the addition of fluids from the skin.

I tried whether pressure applied to the wound, as the first drop was coming out, had the same effect. The wounds were made into the pulps of the fingers, which were firmly squeezed between the finger and thumb of the other hand, immediately after the puncture had been made. The following is the comparison/

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(1) Lancet. Jan.11th 1908.
(2) Bull.et mém Soc. méd.d.hôp. Par. 1901

comparison between the coagulation times of drops of blood obtained without and with pressure.

T.A.

WITHOUT PRESSURE		WITH PRESSURE	
min.	sec.	min.	sec.
7	25	7	10
8	45	8	10
8	15	7	35
8	15	8	5
7	35	7	40
7	45	8	45
7	30	7	50
9	5	8	25

The average coagulation time, without pressure, is 8 min.$4\frac{3}{8}$ sec. and with pressure, is 8 min. 5 sec., so that pressure appears to make no difference.

5. THE RATE OF FLOW OF BLOOD FROM THE WOUND.

The size of the wound, and the vascular condition of the part, may be such that the rate of flow is so slow, that it is necessary to wait for a minute/

minute or more, before a sufficiently large drop has collected. This leads to variations in the time of exposure of the drop to the air, and to the wound and surrounding skin surface. I therefore tried to see whether such variations had any effect on the coagulation time. The results obtained when the blood was taken in the usual way, so that the time between the puncture and the introduction of the blood into the oil is from 7 to 12 seconds, were compared with those given when the drop of blood was left on the finger for from 50 to 60 seconds.

EXPOSURE TO AIR FOR FROM SEVEN to TWELVE SECONDS.	EXPOSURE TO AIR FOR FROM FIFTY to SIXTY SECONDS.
min.sec.	min.sec.
7 10	8 30
7 45	5 30
7 30	8 15
8 30	8 10
7 40	6 20
7 55	6 40
7 45	7 25
8 15	6 20
8 35	7 0
8 5	8 0
7 30	8 5
6 50	5 50
7 15	8 0

The average time when the exposure to the air/

air was 12 seconds or less, was 7 min.45 sec. The average time when the exposure was from 50 to 60 seconds was 7 min.14 sec. There is thus, on an average, a slight diminution in the coagulation time, when the blood is left on the skin for some time, indicating that exposure to the air has a rather greater effect than the friction against glass, which is the means used in the apparatus to produce coagulation. On comparing the results, it will be seen that the times are less uniform with the long exposure to air.

This is clearly seen, if they are tabulated according to the number of seconds they are above, or below, the average time.

EXPOSURE TO AIR FOR FROM SEVEN to TWELVE SECONDS.	EXPOSURE TO AIR FOR FROM FIFTY to SIXTY SECONDS.
-35 seconds	+ 76 seconds
± 0 "	-104 "
-15 "	+ 61 "
+45 "	+ 56 "
+ 5 "	- 54 "
+10 "	- 34 "
± 0 "	+ 11 "
+30 "	- 54 "
+50 "	- 14 "
+20 "	+ 46 "
-15 "	+ 51 "
-55 "	- 84 "
-30 "	+ 46 "

Thus/

Thus the short exposure to air, gives results with an average variation from the mean of 24 seconds, while with the long exposure, it is 53 seconds.

This is what might have been expected, for the air of a room has an inconstant effect on the blood mainly I think because its temperature is so variable. The above results were obtained in a room, in which the windows were wide open, and there were, no doubt, now and then, currents of cold air passing over the blood as it lay on the finger. It is possible also that differences in the amount of dust which settled on the drop, might account for some part of the variations.

6. THE PARTS OF THE BODY FROM WHICH THE BLOOD IS TAKEN.

The fingers are the best parts from which to obtain the blood, because in them a constant rate of flow can be ensured, by employing temporary congestion. There is no difference in the coagulation time of blood from different fingers. Blood from the lobes of the ears, if obtained under the same conditions/

conditions, also coagulates in the same time, and there is no reason to suppose that the same result would not be found, with blood from any part of the surface of the body if a drop of the right size could be obtained without waiting. On account, however, of the thickness of the skin and the poor vascular supply of many parts, this is not practicable.

7. THE POINT IN A HAEMORRHAGE AT WHICH A SPECIMEN OF THE BLOOD SHOULD BE TAKEN.

The following experiment shows that blood taken from a haemorrhage at different times after the infliction of a small puncture wound, coagulates more and more quickly as time passes. Deep punctures were made and the blood was allowed to drip from the finger. The first blood to appear was taken, some from the mid-point in the haemorrhage and some towards the end. My method is not applicable to such a haemorrhage. A spherical drop, not a stream of blood is necessary, and I also found that, as time passed the blood became so agglutinated, that its free flow in the apparatus was interfered with. So I used McGowan's method, which I have modified, by the/

the addition of an apparatus intended to keep the blood at a constant temperature.

T.A.

Temperature of water = 18° C.

	Min.	Sec.
First Blood to appear.	7	15
Blood after one minute's flow.	5	15
Blood after two minute's flow.	3	40
First blood to appear.	6	45
Blood after one minute's flow.	5	15
Blood after two minute's flow.	3	45
First Blood to appear.	7	45
Blood after one minute's flow.	4	0
Blood after two minute's flow.	2	45
First blood to appear.	9	15
Blood after one minute's flow	5	40
First blood to appear.	?	
Blood after one minute's flow.	5	20
Blood after two minute's flow.	4	50

The coagulation time is seen to progressively diminish. This I think is probably due to more and more fibrin ferment forming and collecting about the wound and surrounding skin.

If the amount of this diminution could be shown to be constant, there is no reason why the specimen of blood should not be taken at any fixed point in a haemorrhage; but in all probability it is not constant, and, in any case, it is much more convenient to make a small puncture which will yield only one drop.

THE CONDITION OF THE SKIN-SURFACE SURROUNDING THE WOUND.

(1) THE EFFECT OF THE CONTACT OF THE BLOOD WITH THE SKIN.

This is negligable, if the blood is removed from it within a reasonable time. I made a number of observations to see if its influence could be estimated. Lanoline was rubbed into the finger which was then pricked. The blood ran out onto the surface of the lanoline, so that it did not touch the skin at all. Lanoline being an oily substance may be expected to have no effect on the coagulation time./

time. No constant difference was found from the coagulation time of blood which had been touching the skin. The times when lanoline was used, were sometimes variable because some of the lanoline remained attached to the surface of the drop which was used.

The fact that contact with the skin does not hasten coagulation so much as does contact with air, can be readily shown, by pricking the finger so that a fairly large drop collects. After some time, it will be found, that there is a film of coagulated blood over all the surface of the drop which is touched by the air, while none can be demonstrated next the skin.

A little later a film of coagulum will be found lying on the skin, while the centre of the drop still remains fluid and is the last part to coagulate

(2) EFFECT OF CONTAMINATION OF THE SKIN SURFACE WITH RECENTLY SHED BLOOD.

This has a marked effect on the coagulation time. A drop of blood was smeared over the finger and after the lapse of five or six minutes the finger was punctured, and the freshly issuing drop taken, as it flowed out over the film of old blood. The coagulation times obtained, when the smear was still wet/

wet, and when it was dry, are compared with the times taken by blood obtained from a skin surface free from blood.

Skin free from blood.	Skin covered with a film of wet blood	Skin covered with a film of dry blood.
min.sec.	min.sec.	min.sec.
6 40	4 50	6 35
7 50	4 20	3 0
7 55	3 40	6 5

The blood which had been in contact with skin, smeared with still fluid blood shed some time previously, showed a marked reduction in the time. In other experiments also I have invariably found this to be so. On the other hand when the old blood has dried up, the amount of diminution of the time varies irregularly, and sometimes does not occur at all.

Traces of blood from previous wounds may, therefore, lead to serious error. This applies, of course, not only to the skin but to any parts of the apparatus which the blood touches.

The methods of cleansing the skin which are employed are washing with soap and water or with water/

water, alcohol and ether. These cannot be considered as absolutely trustworthy, Fibrin ferment is not destroyed by the addition of water or of alcohol, nor, so far as I know, does ether render it inert.

The only safe way would appear to be to raise it to a temperature above 65° C. (1) The way in which this can be done is described in Section III.

CONCLUSIONS AS TO THE METHOD OF OBTAINING THE BLOOD.

1. The amount of influence which the wound has in determining the coagulation time is slight, in comparison with the effect of the environment of the blood, after it has issued from the wound.
2. The depth of the puncture has no direct effect.
3. Congestion of the part, in which the wound is made, does not alter the time.
4. Pressure near the wound has no direct influence on the time.
5. The rate of flow is of importance only, in so far as it increases or diminishes the time of exposure/

(1) Fibrin Ferment is destroyed at this temperature Schäfers Text book of Physiology Vol.i p.168.

exposure of the drop of blood to the air and skin, and in so far as the depth of the wound, or the state of congestion of the part, or pressure near the wound have an influence on the rate of flow, they have an indirect effect on the coagulation time.

6. The fingers are the best places from which to obtain the blood. There is no evidence to show that blood obtained from any part of the skin surface under the same conditions would not coagulate in the same time.
7. Blood flowing from small puncture wounds, coagulates more and more quickly as time passes,
8. The contact of the blood with the skin for a few seconds has no appreciable effect.

Contamination of the skin surface into which the wound is made, with recent blood, leads to a considerable diminution of the time. Traces of preformed fibrin-ferment are best destroyed by heat.

2. THE MAINTENANCE OF THE BLOOD AT A CONSTANT TEMPERATURE IN ALL COMPARATIVE OBSERVATIONS.

The value of any method is mainly determined by its success, or want of success, in maintaining the blood at a constant temperature, after its removal from the body.

Where no attention is paid to differences of temperature the method is practically worthless for the influence of even the slight variations which occur in rooms,wards, or laboratories is so great as to make comparative observations valueless.

This is in direct contradiction to the conclusions of the originators of some of the methods, who, while admitting the importance of large variations, believe that slight ones have so little effect that they may for practical purposes, be neglected. No one of them, however, has brought forward any experimental proof of this assumption.

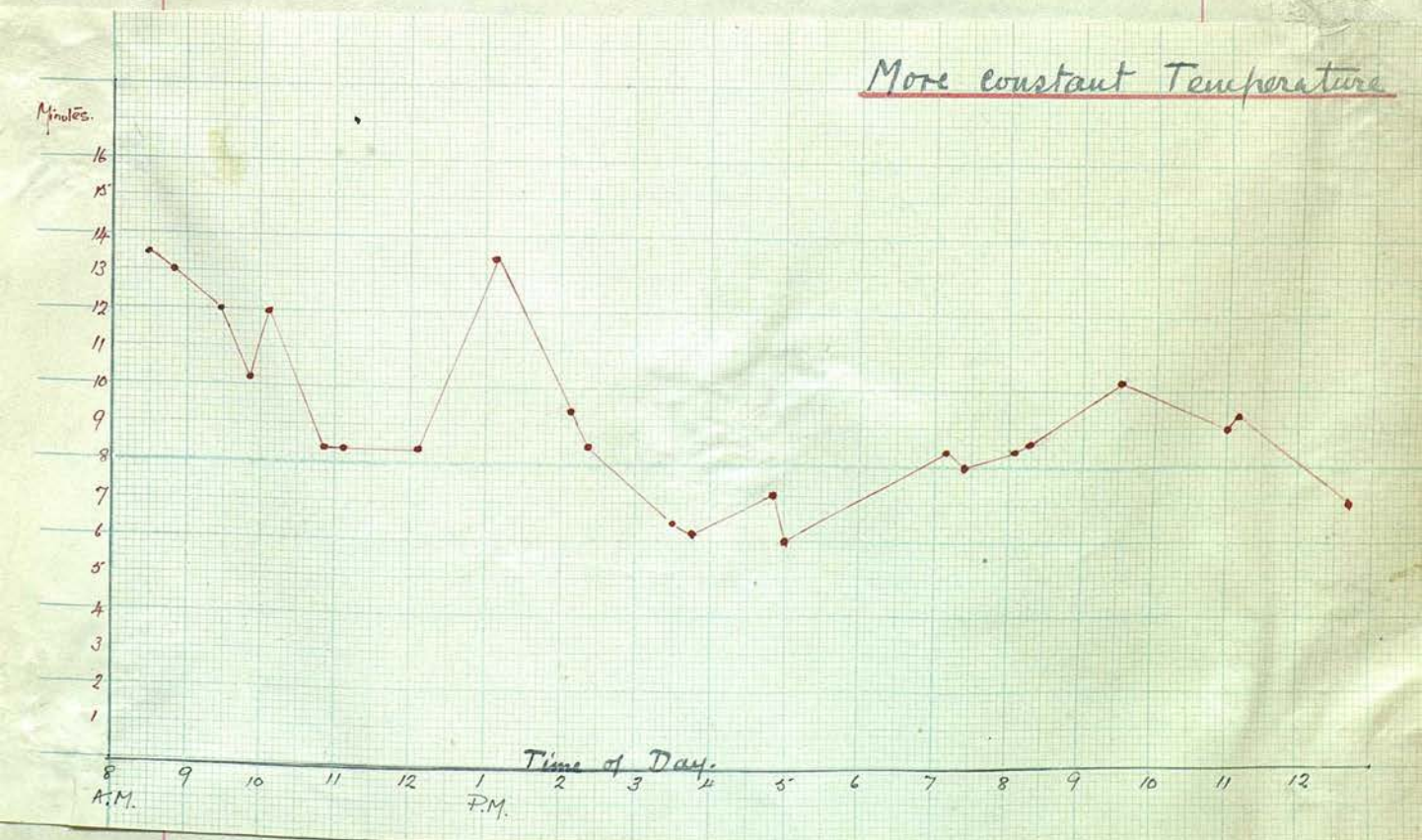
The extraordinary variations in the coagulation times obtained by the use of their methods are to a great extent due to variations in the temperature.

The following is a chart showing consecutive coagulation times, taken in a room in which the temperature/

temperature never varied so much as to make the room noticeably cold or hot. McGowan's method which I think, in other respects, is the most reliable of all the published methods, except Sabrazès was used. The red dots indicate the coagulation times and the black ones the temperature of the air of the room, taken by a thermometer placed close to the apparatus.

The time of day is given in the lower horizontal line and the coagulation time in minutes on the vertical line.

The amount of variation in the time is very great and the curve of coagulation, though complicated by experimental error due to other causes, is seen roughly to run in the opposite direction to the curve of temperature. That this wide variation in the time is mainly due to the temperature variation is seen in the following chart. The times given in it are not so variable because an attempt was made to maintain the tubes at a constant temperature of 15·5° C. The method adopted was simply to move the thermometer to different positions, between a fire and an open window, until a place was found where it recorded 15·5° C. and the experiments were conducted in this situation.



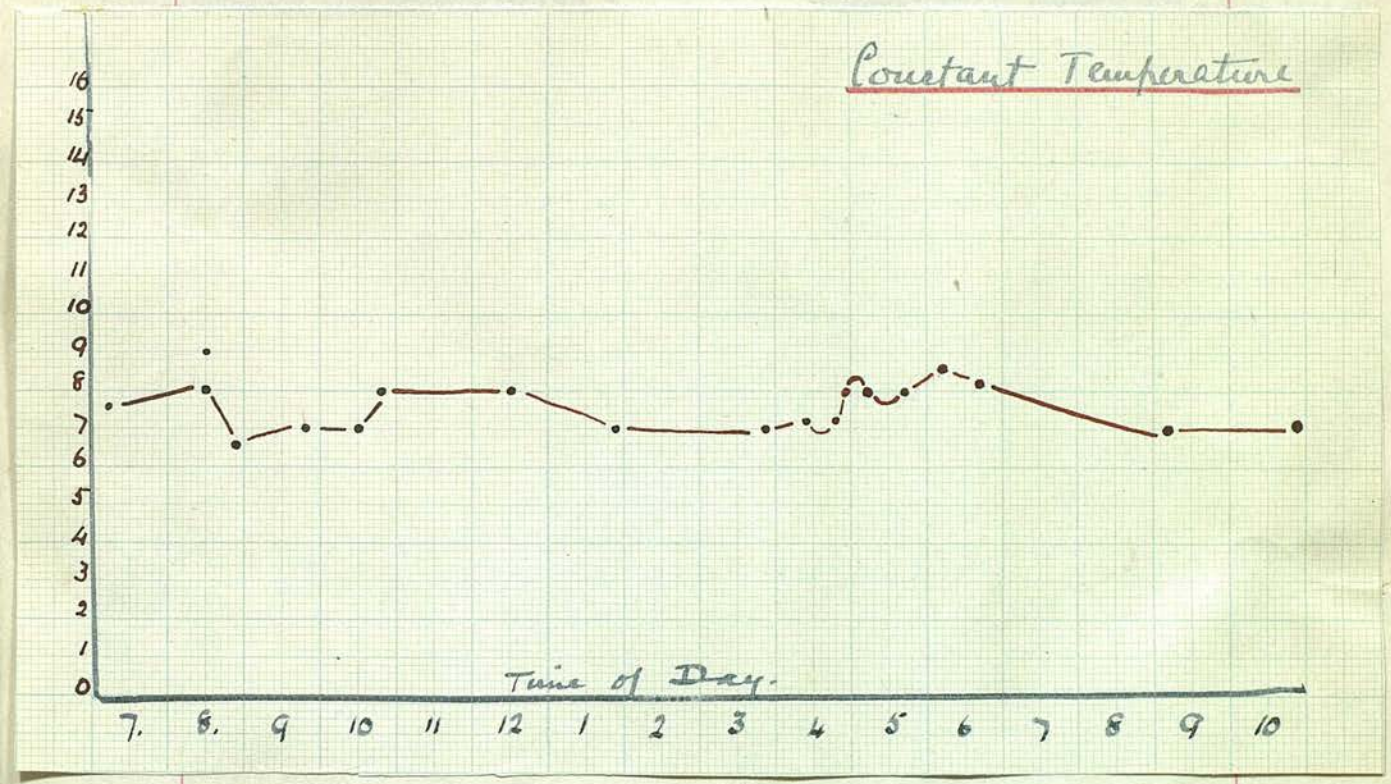
In the next chart the times were taken by the same method modified by keeping the tubes in the apparatus described under "A modification of McGowan's method" in Section ii.

In this modification the temperature is kept still more constant, and it will be seen that there is a corresponding diminution in the amount of variation in the coagulation time.

The coagulation times in the following chart were taken by my method in which the tempera-

ture/

temperature is kept very nearly absolutely constant.



Here the variation is still less and is due. I think to experimental error (see Daily Variations in the Coagulation Time. Section IV)

The/

The actual, or very nearly the actual amount of difference which the temperature makes on the coagulation time is seen in the table given below. Each coagulation time given, is the average of two or more estimations so that the amount of experimental error is diminished.

EFFECT OF SLIGHT VARIATIONS IN THE TEMPERATURE ON THE COAGULATION TIME.

TEMPERATURE.	AVERAGE COAGULATION TIME.
	min. sec.
13·5° C.	14·32½
14·5 "	12·58¾
15·5 "	11·46½
16·5 "	10·10
17·5 "	8·27
18·5 "	7·34½
18·5 "	6·2½
20·5 "	5·22½

These temperatures are within the amount of variation often found in laboratories or wards in this country. The effect of very high and very low temperatures is given in Section IV.

3. THE CONTACT OF THE BLOOD WITH THE SAME AMOUNT AND KIND OF FOREIGN BODY IN EACH OBSERVATION.

A foreign body as regards blood, may be defined as anything which hastens or retards the coagulation time.

Besides the intact endothelial lining of the vascular system, there is only one class of substances which may be said not to act as foreign bodies i.e. the oils. I do not think that the ordinary commercial oils can, strictly speaking, be excluded from classification as foreign bodies, for I found that the coagulation time of blood surrounded from the moment it issued from the wound by "motor spirit" was less than the time taken when ordinary paraffin was used. Paraffin gave a time of from 70 to 80 minutes, while with Pratt's Motor Spirit it was 50 to 60 minutes. Nevertheless, this action as foreign bodies is so slight, in comparison with that of other substances, that they may be considered as having practically no effect at all.

This fact may be utilised to estimate the effect/

effect of foreign bodies on the coagulation time. For by placing a drop of blood in partial contact with a foreign body and surrounding it by oil, the complicating effect of other substances is excluded, and the resulting coagulation time gives an indication of the influence of that particular foreign body.

Of course, blood obtained from a wound will always coagulate even if contact with foreign bodies is entirely prevented, for in its passage from the vessels to the surface of the skin it receives the stimulus which sets in motion the process of coagulation. Even when the blood is taken directly from a vessel without contact with the tissues, it is not possible with mammalian blood to entirely prevent coagulation, although Bordet and Gargon (1) by inserting paraffin canulas into the carotids of dogs and rabbits and receiving the blood in paraffin lined vessels have very nearly succeeded.(1)

In all the different methods, excluding Vierordt's, the only foreign bodies used are air, glass and platinum wire. The following experiments show/

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(1) Ann. Inst. Pasteur. 1901.

show the different times which are obtained, when coagulation is due to the action of the wound only, to the wound and contact with air, to the wound and contact with glass, and to the wound, glass and air.

As will be seen the method which is used of determining that coagulation had taken place is a very rough one, but it is amply sufficient to indicate the wide differences in the coagulation times obtained under the influence of the different foreign bodies.

1. EFFECT OF THE WOUND.

Five small basins were immersed in a vessel containing melted block paraffin. When they were taken out the film of liquid paraffin adhering to them became solid and they were thus covered with a smooth layer of solid paraffin. They were immediately filled with the ordinary commercial paraffin used for burning in lamps. Into each basin a finger was put and was pricked while immersed under the oil. When the drop of blood had attained a certain size, it detached itself from the finger and floated to the bottom of the vessel which was prevented from acting as a foreign body by the covering of paraffin wax. The time at which the blood was drawn was noted in each case. After waiting for some time the oil was poured/

poured off the first basin and the drop of blood was touched with filter paper. If it all ran into the paper it was considered not to be coagulated. This was repeated at intervals of about 20 minutes until a drop was found which the filter paper did not all absorb, a mass of fibrin being left. This was considered as indicating coagulation.

EFFECT OF THE WOUND.

CONTACT WITH NO FOREIGN BODIES AFTER LEAVING THE WOUND.

TIME OF PRICKING FINGER.	TIME OF TESTING FOR COAGULATION.	TIME BETWEEN THESE TWO ACTIONS.	RESULT.
hrs.min.sec.	hrs.min.sec.	min.	
3 43 30 p.m.	4 13 30	= 30	= No Coagulation
3 49 0	4 34 0	= 45	= "
3 56 0	4 56 0	= 60	= "
4 3 0	5 13 0	= 70	= "
4 8 0	5 28 0	= 80	= Coagulation

Conclusion:- Blood protected from the action of foreign bodies from the time it left the wound, takes from 70 to 80 minutes to coagulate.

2./

2. BLOOD EXPOSED TO AIR ONLY AFTER LEAVING THE WOUND.

Five glass slides were coated with a thin film of paraffin wax. A drop of blood placed on each was exposed to the air. Coagulation was tested for in the same way.

TIME OF PRICKING FINGER.	TIME OF TESTING FOR COAGULATION		TIME BETWEEN THESE TWO ACTIONS.	RESULT.
hrs.min.sec.	hrs.min.sec.		min.	
4 37 30	4 47 30	=	10	= No Coag.
4 41 0	5 0 0	=	19	= Coag.
4 42 0	5 0 0	=	18	= "
4 43 0	5 0 0	=	17	= "
4 44 0	5 0 0	=	16	= "

Conclusion:- Blood exposed to the action of air coagulates in from 10 to 16 minutes. Only the part of the drop which was in contact with the air coagulated, there was no coagulum next the paraffin.

3./

3. BLOOD EXPOSED TO GLASS ONLY AFTER LEAVING THE WOUND.

Five glass slides were carefully cleaned with water, alcohol and ether. Five small basins were filled brimful of oil. A drop of blood was placed on each slide and these were immediately put blood downwards into the basins of oil, so that the whole drop was surrounded with oil, with the exception of that part of the blood, which was in contact with the glass.

TIME OF PRICKING FINGER.	TIME OF TESTING FOR COAGULATION.		TIME BETWEEN THESE TWO ACTIONS.		RESULT.
hrs.min.sec.	hrs.min.sec.		min.		
3 12 0	3 32 0	=	20	=	No Coag.
3 13 30	3 43 30	=	30	=	Coag.
3 15 0	3 44 0	=	29	=	"
3 16 30	3 44 0	=	28	=	"
3 18 0	3 44 0	=	26	=	"

Conclusion:- Blood in contact with glass, coagulates in from 20 to 26 minutes.

In each case where coagulation was found to have taken place, there was found a thin film of coagulum occupying the space where the drop had been in contact with the glass, exactly the opposite of what occurred when the drop was exposed to air.

4./

4. BLOOD IN CONTACT WITH BOTH AIR AND GLASS AFTER LEAVING THE WOUND.

Five glass slides were carefully cleaned. A drop of blood was taken on each, and they were then exposed to air

TIME OF PRICKING FINGER.	TIME OF TESTING FOR COAGULATION.	TIME BE-TWEEN THESE TWO ACTIONS.	RESULT.
hrs.min.sec.	hrs.min.sec.	min.	
4 53 0	5 1 0	8	Coag.
4 54 0	5 1 0	7	"
4 54 45	5 1 0	6¼	"
4 55 15	5 1 0	5¾	"
4 56 0	5 1 0	5	"

Conclusion:- Blood exposed to both air and glass coagulates in less than 5 minutes.

In every case the only demonstrable coagulum was the part exposed to the air. The blood in the centre and that touching the glass appeared to be still uncoagulated.

5./

5. COMPARISON OF RESULTS.

1. Blood exposed to no foreign body after coming from the wound coagulates in 70 - 80 min.

2. Blood exposed to glass coagulates in 20 - 26 min.

3. Blood exposed to air coagulates in 10 - 16 min.

4. Blood exposed to glass and air in less than 5 min.

The fact that not only the amount but the kind of foreign body to which the blood is exposed is of importance is clearly shown by the above experiments, and indicates that any method which is inconstant in this respect is bound to give erroneous results. The most striking point is the great effect of contact with air, about twice as great as that with glass. In all the methods the blood is exposed for a longer or shorter time to air, in some of them only during the short time between the drawing of the blood and its introduction into the apparatus, but these results show the importance of making this time as uniform as possible

4. A CLEAR AND DEFINITE END-POINT WHICH SHALL BE THE RESULT OF THE SAME DEGREE OF COAGULATION IN EACH EXPERIMENT.

A method of estimating the coagulation time is devised with the aim of demonstrating, that after a certain time the blood undergoes a degree of change, which leads to a corresponding difference in its behaviour to its surroundings.

This indication of difference is the End-Point of the method. When it has been observed the blood is said to be "coagulated", and the coagulation time, is the time elapsing between the drawing of the blood and the appearance of the end-point.

The methods may be divided into two classes, according to the end-point which they adopt.

In the first class, the development of fibrin in quantity sufficient to be recognised by the unaided eye is taken, and the methods aim at showing this fibrin clearly.

In this class are Wright's method, Sabrazès' Mc.Gowan's method, and Bürker's method.

In Wright's method, the end-point is the appearance/

appearance inrecognisable amount of fibrin in blood, when it is blown out of a capillary tube, after the lapse of a certain interval of time.

In Sabrazès' and Mc.Cowan's method, it is the first appearance of a thread of fibrin, when capillary tubes which have been filled with blood are broken, and the fractured ends drawn slowly apart. In Bürker's, it is the first appearance of a thread of fibrin, when fine glass rods are drawn through a mixture of blood and water.

In the second class the indication that the blood has coagulated is an indirect one. Fibrin is not seen, but it is judged to be present from changes in the behaviour of the blood, which are ascribed to the formation of fibrin.

Thus in Vierordts' method, the blood is said to be coagulated, when it no longer adheres to a horse-hair which lies in it.

In Brodie & Russell's method the end point is the time, when a drop of blood attains such a degree of cohesion as to have its margin indented by a blast of air directed on to it.

In Milian's method and its modifications, in Buckmaster's and in my method, the end-point is taken/

taken as the time when the blood ceases to flow under the influence of the same power which caused it to flow before.

In Biffi's and in Hingston Fox's methods, it is the time when the blood, on being put into water, no longer diffuses but remains as a solid mass.

These different end-points will be discussed in detail in connection with each method; but, in the meantime, it will be seen that all in the second class depend on the formation of such an amount of fibrin in the blood, as to cause it to react in a different manner than it did before, to the environment supplied by the method.

1. THE DIRECT MACROSCOPICAL DETECTION OF FIBRIN AS A MEANS OF DETERMINING THAT A CERTAIN DEGREE OF COAGULATION HAS BEEN ATTAINED.

I do not wish to discuss here the relative merits of Wright's or Sabrazès' methods of indicating, to the eye the presence of fibrin in blood, but merely the general question, as to whether or not, granted that you have a good way of showing its presence, the appearance of fibrin which can be seen, is a constant and reliable sign of a certain degree of coagulation.

Coagulation/

Coagulation is a gradual process. It probably commences whenever the blood has left the vessels.

Buckmaster (1) says he has demonstrated fibrin threads in films of blood 10 seconds after it has been drawn, although the blood shows no other signs of coagulation.

I have found on touching drops of blood which have been for half a minute or so on the skin with a piece of glass that a fine thread which is either fibrin or a substance indistinguishable from it, can sometimes be seen stretching from the drop to the glass, when it is withdrawn a little from it. These drops were perfectly fluid, and differed apparently in no way from other drops, from which I did not happen to succeed in drawing a fibrin thread.

In this connection, the following extract from Mann's Chemistry of the Proteids 1906 p.382 is interesting.

"Ramsden, in a paper not yet published, states that fibrinogen-solutions, free from fibrin ferment, can be made to yield 'mechanical surface aggregates' indistinguishable from typical fibrin, and/

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(1) "Science Progress".

and that fibrinogen, mechanically produced fibrin, and ferment-produced fibrin, have the same heat coagulation temperature 53° - 58°."

These two facts, first, that shortly after the blood has been drawn and before it shows any other signs of coagulation, fibrin threads can sometimes be seen, and second, that fibrin may be produced by purely physical agencies, show that the first appearance of fibrin is a very variable thing, depending more on the physical agencies used to demonstrate it, than on the condition of the blood as regards coagulation.

But it may be said that the physical agencies used in Wright's, Sabrazès' and Bürker's methods, are not such as to mechanically produce fibrin, and that although it may be admitted that fibrin is present, to some extent, from the commencement and goes on increasing, yet it is only when a certain amount has been produced, that enough is present under the circumstances of the method employed to yield a fibrin thread, or a mass of fibrin which can be seen.

As regards the mechanical production of fibrin, if the fibrin which is sometimes seen, by touching/

touching drops of fresh uncoagulated blood with glass, is mechanically produced, then it is extremely probable that Sabrazès' and Bürker's method in which the end-point is the first appearance of the fine thread of fibrin, will sometimes yield mechanical fibrin, for the physical agencies are the same in both cases.

If, on the other hand, it is not mechanical but indicates fibrin-ferment coagulation of the blood, it shows how uncertain a test of the extent of such coagulation, a fibrin thread is.

With Wright's method there is not such an opportunity for the mechanical production of fibrin.

As regards the second point, that in spite of the above demonstration of the inconstancy in the time of appearance of fibrin, that nevertheless under the physical conditions of the methods it is only when a certain amount of fibrin has formed that it can be demonstrated, it must be said, that these methods cannot be regarded as an accurate quantitative test of the amount of fibrin produced.

In Sabrazès' and Bürker's methods, the finest thread which can be seen, is taken as evidence of coagulation and as has been shown, this does not presuppose any fixed amount of coagulation.

In/

In Wright's method, the first appearance of a mass of fibrin in the little pool of blood which has been blown out of the capillary tube is taken. It is obvious that this appearance does not depend altogether on the amount of coagulation, but rather on the physical circumstances which lead to fibrin becoming visible at all.

Fibrin is deposited in an amorphous and invisible condition, and it is only under the influence of various mechanical stresses and strains, that it acquires the appearance of a fibrin thread or of a mass of fibrin. These physical circumstances are impossible to control. Minute differences in the smoothness or form of the capillary tubes, slight differences in surface tension, the manner in which the blood is blown out of the tube and the surface on which it is received, are no doubt factors in production of the form which the fibrin assumes; but it is impossible to control these factors. Besides, there may be conditions of the blood itself, which may sometimes favour the production of fibrin threads and masses, and at other times tend to maintain it, in a more or less amorphous condition.

The ultimate test of a method however must be practical not theoretical. This will be gone into fully later, but it may be mentioned here that Wright's/

Wright's latest method appears from the results obtained by the only observer (1) who, so far as I know, has given the method a thorough trial, to be extremely fallacious.

He compared the coagulation times of normal people with those of epileptics, not, however, with any idea of criticising the method. The great variations which he finds in successive estimation in the same individual he ascribes to variations in the coagulation time of the blood, and not to faults in the technique of the method.

He says, "As illustrating the very great variation in coagulation, it may be stated that from three successive pricks in the same individual in different fingers, the blood, in both control and epileptic cases, often showed a difference in the time of over two minutes, and, in all my observations, I only, on three occasions, found the same coagulation rate in the blood from three successive pricks."

He made considerably over a thousand estimations and his average time was 160 seconds, so that a variation of 120 seconds in the same individual, which he says he often got, can with certainty be ascribed not to the blood but to the method.

2/

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(1) J.Turner, Journal of Mental Science Jan. & Oct. 1907.

2. THE INDIRECT METHODS OF DETERMINING THAT A CERTAIN DEGREE OF COAGULATION HAS BEEN ATTAINED.

These, also, are supposed to depend on the formation of fibrin, but not necessarily of such fibrin as can be seen.

It is sufficient that the fluidity of the blood is so diminished, that a definite alteration in its behaviour to its surroundings may be observed.

Thus the blood may become so solid, that it no longer adheres to foreign bodies (1), that a drop on a slide ceases to show a change of outline when the slide is tilted (2), that a drop has its edge indented when a stream of air is directed against it (3), that a film of blood on a platinum loop ceases to flow when the loop is placed in a vertical position (4), that the blood no longer diffuses when it is put into water (5), that the blood ceases to flow round under the influence of a stream of oil playing on it (6).

As/

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(1). Vierordt's method.
(2). Milian's "
(3). Brodie & Russell's method.
(4). Buckmaster's method.
(5). Biffi's and Hingston Fox's methods.
(6). My method.

As regards the general value of these indirect methods, I think there is one point which must be considered.

It is taken for granted that when the blood loses to some extent its fluidity and gives rise in this way to the changes which are taken as the end-points of these methods, that this is due to the formation of fibrin. But it is possible, that in some cases the same result may be brought about by the simple agglutination of the corpuscles without coagulation, for this would also lead to a diminution in the fluidity of the blood, and it is difficult to see how the two could be distinguished. The blood becomes agglutinated whenever it ceases to flow.

When a drop of blood, which has just appeared on the skin is at once introduced into oil which is moving in such a way as to tend to make the blood flow round, and the pressure of oil is low, the agglutination of the blood is clearly seen. The blood flows slowly and a little jerkily, and the corpuscles can be seen stuck together in clumps.

When the pressure of the oil is raised, the agglutination is broken up, the corpuscles stream round, each one separate from the other, and once the agglutination/

agglutination has been overcome only half the pressure of oil is necessary, to keep the blood flowing, and for some time it will continue.

Then agglutination can be seen to commence again, the corpuscles sticking together, first in twos and threes, then in larger and larger clumps until the blood becomes stationary. If the pressure of the oil be again raised, it will be seen that now there is a condition not only of agglutination, but also of coagulation, for the higher pressure will wash some of the corpuscles out of the clot and a reticulum of fibrin becomes visible in which the corpuscles are enmeshed.

If instead of taking the blood as soon as it has come from the wound, it be left exposed to the air for two or three minutes, it will be found that, however high the pressure of the oil be raised, the agglutination will still persist and the blood will not flow in the smooth manner in which it moved before, but moves slowly and jerkily and soon stops, although the blood has not coagulated. Agglutination is therefore progressive.

It is thus seen that agglutination is quite distinct/

distinct from coagulation, which it may nevertheless closely simulate, so that in some circumstances it may lead to error.

This objection would not, however, apply to those methods in which the end-point is the non-diffusibility of blood in water. No amount of agglutination could lead to this. But there are other reasons why this is not a good end-point.

No end-point which has yet been suggested can be considered free from fallacy. They all depend too much on the recognition of more or less inconstant physical changes in the blood, which are the result of the chemical change of fibrinogen into fibrin.

A method of estimating the quantity of fibrin present, after a given time, in a small quantity of blood, would be the most direct method, but this is not as yet possible.

S E C T I O N I I .

SECTION II.

THE METHODS OF ESTIMATING THE COAGULATION TIME OF THE BLOOD IN MAN.

Instead of discussing these methods in chronological order, it is more convenient to arrange them according to the end-points, or changes in the blood indicative of coagulation which they adopt.

1. METHODS WHICH TAKE THE FIRST APPEARANCE OF A VISIBLE THREAD OR MASS OF FIBRIN AS THEIR END-POINT.

Wright's Method

Bürker's Method

Sabrazes' Method

McGowan's Method

2. METHODS WHICH TAKE CHANGES IN THE CONTOUR OF THE SURFACE OF THE BLOOD AS THEIR END-POINT.

1. Heyem's Method

2. Milian's Method

   a. Bezancon and Labbe's modification
   b. Hinman and Sladen's modification

3./

3. Brodie & Russell's Method.

a. The Pratt-Grützner modification
b. Bogg's modification

3. METHODS WHICH TAKE THE NON-DIFFUSIBILITY OF THE BLOOD IN WATER AS THEIR END-POINT.

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1. Biffi's Method
2. Hingston Fox's Method

4. A METHOD WHICH TAKES THE FACT THAT THE BLOOD, WHEN IT HAS ATTAINED A CERTAIN DEGREE OF COAGULATION, NO LONGER ADHERES TO A HORSE-HAIR INTRODUCED INTO IT, AS ITS END-POINT.

Vierordt's Method.

5. METHODS WHICH TAKE AS THEIR END-POINT THE MOMENT WHEN FLOWING BLOOD BECOMES STATIONARY.

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1. Buckmaster's Method
2. Author's Method

(described in Section 3)

Each of these methods is described as shortly as possible, along with any modifications which others may/

may have made on them. Any results which may have been published by their authors, or by others who have used them are then shortly stated.

The practical advantages and disadvantages of the method are then discussed, from the point of view of the conclusions arrived at in section I; and, where possible these are illustrated by results which I have obtained in using it. I am much indebted to Mr W.R.Addis for the diagrams of apparatus.

1. METHODS/

1. METHODS WHICH TAKE THE FIRST APPEARANCE OF A VISIBLE THREAD OR MASS OF FIBRIN AS THEIR END-POINT.

WRIGHT'S METHODS.

DESCRIPTION OF METHOD.

Wright first published his method in 1893. (1.) It then consisted of aspirating 5 cm. of blood into capillary tubes with a bore of about .25 mm. The time of filling each tube was noted. The blood was then blown out of each tube in succession, until one is reached from which the blood cannot be blown. This gives the coagulation time.

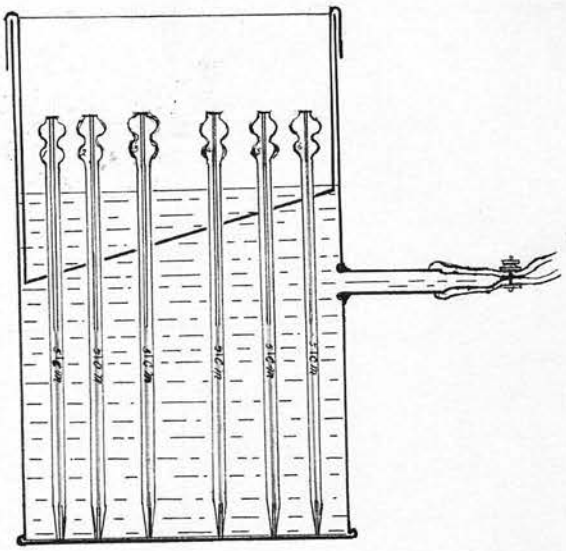
At this time he recommended that the tubes should be filled from the same puncture, wiping away each drop after filling a tube, and expressing another drop for the next tube.

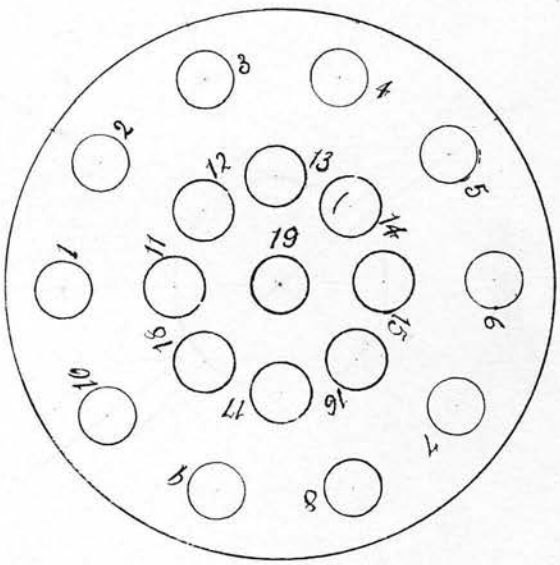
In/

(1). Brit.Med.Journal 1893,II.223.

Wrights Method in 1897.

Shows coagulation tubes filled with blood in position in the can





In 1894 (1.) he modified it in several ways. The blood should be aspirated a little way up the tube, so as to prevent dessication at the end. The tubes should be warmed to blood heat before filling, and kept at that temperature after filling, by slipping them into the pockets of a flannel binder, which encircles a tin of water at 37°.C.

Later in the same year (2.) he recommended a temperature of 18·5°, as the coagulation times obtained thus were longer.

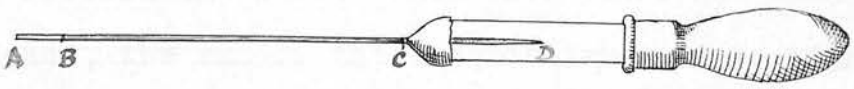
In 1897 (3.) he changed the method of keeping the tubes at a constant temperature. A can of water at 18·5°C. was used, into which the tubes were put end downwards when filled.

In 1902 (4.) with the object of making the method less expensive, he described a method of calibrating capillary glass tubes with mercury.

Later in 1902 (5.) he published an article, in which he modified the method of taking the blood. The blood was no longer to be expressed from one puncture, but each tube was to be filled from a separate/

(1.) Brit.Med.Journal I.237
(2.) Brit.Med.Journal II.57.
(3.) Lancet I.303.
(4.) Lancet 1902 II.11.
(5.) Lancet 1902 II. 1531.

separate prick. The end-point was also changed, from the time when it was impossible to blow out the blood, to the time when a coagulum was first seen.



In 1905 he gives the latest changes in the method. The above is an illustration of the tube. B C is standardised by the aid of Hg and an automatic pipette, so as to contain 5 cubic millimetres in a length of 5 centimetres. This segment holds the blood. C D is filled with Hg which blocks the hair fine orifice as soon as the blood has entered B C., A.B. is filled with air to prevent the water of the bath running into the blood.

" A drop of blood is obtained by pricking the side of the finger near the nail, and applying very gentle pressure to the finger pulp. Blood having thus been obtained, a coagulation tube, which has been furnished with a tightly fitting teat, and which has been primed with the appropriate quantity of Hg. is taken in hand. After the column of Hg. has been driven down by pressing on the teat until its upper border comes level with the distal calibration mark, the extremity of the coagulation tube is introduced into the drop of blood on the finger. When the pressure on the teat is relaxed, the blood enters the tube/

Tube. Its inflow is arrested by pressure on the teat, as soon as the end of the column of Hg. which is in advance of the blood, has come level with the proximal calibrating mark. When this point has been reached, the end of the coagulation tube is withdrawn from the drop of blood on the finger, and the Hg. is allowed to go home to the extremity of the tube, and to engage itself in the hair-fine extremity, preventing further indraught. The column of blood will now be found to have taken up its proper position in the central segment of the tube. Leaving the rubber teat in position, the coagulation tube is now immersed in a water bath at 37°C. placed ready to hand. After the expiration of a minute to a minute and a half, the condition of the blood is tested, by removing the tube from the water bath and making pressure on the teat, in such a manner as to drive out the contained blood, if unclotted, on to a piece of filter paper. When one tube has been tested in this manner, a second is taken in hand and is filled in, from a freshly made puncture. A series of 3 tubes generally suffices for the whole estimation. Where only a single tube is available, it will still be possible to measure the coagulation, by blowing out on to the filter paper, a series of samples of the contents of the tube, returning the tube to the water bath in the intervals".

EXAMPLE/

EXAMPLE OF THE PRACTICAL WORKING OUT OF THE ABOVE METHOD.

Seven coagulation tubes are filled in seriatim, with blood from the finger of a normal man, and are placed in a water bath at 37°C., the times of filling in and testing the tubes being in each case noted.

Tube 1	blown out on to filter paper	after 30 sec	No Clot
2	"	45 "	"
3	"	1 min	"
4	"	1 m.15 sec	"
5	"	1 m.30 sec	"
6	"	1 m.40 sec	Shred of Fibrin
7	"	2 m. 0 sec.	Firm Clot.

Evidence of fibrin formation having been obtained after the expiration of 1 min.45 sec. while no trace of fibrin was found after 1 min.30 sec. the coagulation time of the blood is here taken as 1 min. 37 sec.

2.RESULTS WHICH HAVE BEEN OBTAINED BY SIR A.E. WRIGHT AND OTHERS BY THE USE OF THIS METHOD.

a. WRIGHT.

In 1893 (1) he published a few experiments, which showed a reduction of the coagulation time after calcium. Neuclein had no effect on the time.

In 1894(2) he gave some more results showing a diminution of the time after calcium. He notes that continuous administration of Calcium does not keep the coagulation time short. The inhalation of CO^2 causes a diminution in the time. Citric acid lengthens the time, but citrates and tartrates have no effect.

Rapid respiratory movements diminish the time. Alcohol lengthens the time.

In 1896 (3) he discussed the treatment of haemorrhage and of urticaria by Calcium and gives some coagulation times before and after Calcium, later in 1896 (4) he published an article called "Association of Serous Haemorrhages with Conditions of Diminished Blood Coagulability."

Urticaria, Chilblains, physiological albumenuria, some pleural, ascitic and joint effusions, and certain forms of diarrhoea, are said to show a condition/

(1) Brit. Med.Journal 1893 ii 223.
(2) Do. 1894 ii 57.
(3) Lancet 1896 i 153
(4) Lancet 1896 ii 807.

condition in which the blood takes a long time to coagulate. A few examples of coagulation times in these conditions were given.

(1)In 1897, in "the Pathology and Treatment of Chilblains" he gives eight cases, most of which were improved or cured by Calcium. In each case he took the coagulation time of the blood several times during the treatment, and shows that those cases which were not benifited by the Calcium were these in which little or no diminution of the coagulation time was found.

In 1902 (2) he gives 28 coagulation times in normal men and 28 corresponding estimations of the amount of Calcium in the blood. The method of estimating the amount of calcium was to find what strength of oxylate solution was necessary to render the blood incoagulible. The calcium is expressed in terms of the strength of oxylate solution.

He finds that there is no correspondence between the coagulation time and the amount of calcium

In 1902 (3), in conjunction with Knapp, he showed that there was a diminution of the coagulation time in cases of typhoid, to which he attributes the frequency of thrombosis in that disease. He thinks that the milk diet in these cases is responsible for the/

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(1) Lancet 1897 i 303.
(2) Lancet 1902 ii 1531
(3) Lancet 1902 ii 1460

the short coagulation time.

In 1905 (1), he gives some coagulation times, after the administration of Calcuim, showing that the fullest effect is seen in three quarters of an hour. He also gives three cases, treated with Citric Acid, which showed a lengthening of the coagulation time.

2. MURPHEY & GOULD (2)

They made 300 observations with Wright's 1897 method. In 15% of these no result was arrived at. They come to the conclusion that Brodie & Russells method is the better.

3. ROSS (3)

He gives fourteen cases, in which the coagulation time was diminished after Calcium. He used the 1905 method.

4. COLEMAN (4)

He used Wright's method and Brodie & Russell's method, but preferred the latter and did most of his work with it.

5. SOLIS - COHEN (5)

Made sixty-five observations, in normal and pathological conditions. "The results obtained were all practically negative and were, moreover, unsatisfactory."/

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(1) Lancet 1905 ii 1906.
(2) Boston Med.& Surg. Journal 1904
(3) Lancet 1906 i p.143
(4) Bio-Chemical Journal Vol.ii No.4 1907
(5) University of Penna. Med. Bulletin June 1907.

factory."

6. DOUGLASS (1)

Used Wright's method in comparing the coagulation time of normal and pregnant women and those suffering from albuminuria and eclampsia.

7. WRIGHT & ROSS (2)

In a paper called "The discrimination of Physiological albuminuria from that caused by renal disease" They give coagulation times taken by the 1905 method, before and after calcium, showing a diminution of the coagulation time after the administration of Calcium.

8. NIAS (3)

He showed that Strontium salts diminished the coagulation time. In 1908, he publishes observations on the effect of the Alkaline earths.

He says "In spite of criticisms which have appeared as to the sufficiency of this method, it has proved itself amply adequate for the purpose in hand, very consistent results having been obtained."

9. HINMAN & SLADEN (4)

"The pathological differences and those dependent on technique, in this method, are of about the same relative value, which must confuse the results."

10. TURNER (5)

He made over 1000 observations with the 1905 apparatus. His average time was 160 seconds, and he says/

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(1) Brit. Med. Journal P 709 1904
(2) Lancet ii 1104 1905
(3) Lancet ii 436 1906, Lancet January 1908
(4) John Hopkin's Hospital Bulletin June - July 1907
(5) Journal of Mental Science Jan. & Oct. 1907

says, that he often found differences of 120 seconds in blood taken from successive punctures in the same individual.

DISCUSSIONS/

DISCUSSIONS OF THE RESULTS GIVEN BY WRIGHT'S METHOD. (1)

Although Wright has modified his method so many times it has always remained the same in principle.

The 1905 method is as regards its most important point, i.e. temperature, a reversion to the one described in 1894. In all the others the temperature is 18.5° C.

Forty three estimations were made with the method described in 1897, with the exception that each tube was filled from a separate puncture as he recommends in 1902. In nearly 50% of these only an approximate time was arrived at, because though coagulation was shown to have taken place in one tube in a certain time, yet in another one no coagulation had occurred even after the lapse of a still longer time. For instance coagulation was found to have taken place in one tube after 9 min. and yet in another there was no coagulation after 10 minutes.

This points to the existence of an experimental/

(1) Lancet i 303 1897.

experimental error, which must be attributed to a deficiency in the end-point for the tubes were under identical conditions as regards temperature and contact with foreign bodies.

The Coagulation time of blood taken in successive days from the same individual showed large variations. This was partly due to errors in temperature. It is practically impossible to keep a small tin half full of water at a consistent temperature for from 5 to 10 minutes simply by adding warm water to it. Variations of several degrees occur even when reasonable care is exercised. Besides one is so fully occupied in filling the 6 to 12 tubes and in blowing the blood out from them, that there is very little time to attend to the temperature.

THE LATEST MODIFICATION.

The only essential change is the change of temperature from 18·5° C. to 37° C.

It is certainly better only to use the tubes once as is done in this method for there is then no chance of error from pre-formed fibrin-ferment, but on the other hand it adds very greatly to the time and trouble entailed.

Wright/

Wright suggested that the tubes should be made by the experimenter himself in order that expense might be spared. But very few people can give the time which is necessary. I found that it took a skilled glass-blower practically a whole afternoon to make half a dozen tubes.

Some results which I obtained with this modification in connection with the administration of Calcium are given in Section IV. I have not had much experience of it but in view of the very large number of observations made by Turner (1) and of the results which he has recorded, there is no great necessity for a large number of observations especially as it only differs from the previous modification on the point of temperature. As Hinman and Sladen (2) have said the change of temperature from 18.5 to 37° C. is not an improvement because it makes the coagulation time so short that experimental errors have a much greater influence on the results.

In one way, however, it is of advantage. The time of the experiment being so short the temperature of the water bath remains practically constant.

But/

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(1) Turner. Journal of Mental Science. Jan. and Oct. 1907.
(2) Hinman & Sladen. John Hopkins Hospital Bulletin June - July 1907.

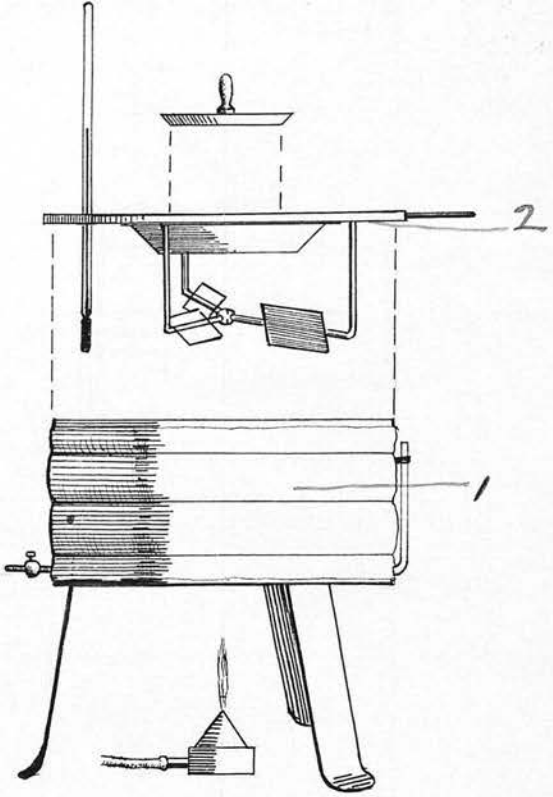
But just because of the shortness of the coagulation time any variation in the length of the period between the time when the blood is drawn and the time when the filled tube is put into the water, must have a considerable influence on the result, for during this period the blood is exposed to room temperature. Wright records times of 30 seconds. I found that I took nearly that time before I could put the tube into the water, for the pipette must be compressed very carefully to prevent the mercury in the tube being driven out. Probably, however, with practice this period might be made shorter and approximately constant but it will always remain as a constant opportunity for error.

The main objection to the method, however, is to be found in the end-point. In the 1897 modification the end-point was the time when the blood could no longer be blown out of the tube. This was a clear and definite phenomenon, but the difficulty was to blow always with the same degree of force.

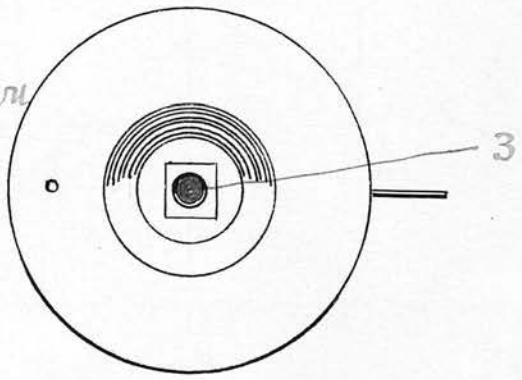
In the 1905 method the end-point is the first appearance of fibrin, when the blood is driven out into filter paper. Now, it is difficult to see/

see fibrin in the expelled drop unless it is in large amount. In practice therefore one is apt sometimes to spread out the drop in order to see whether any is present or not, or to blow out the blood while moving the tube over the filter paper so that the blood is deposited in a line. It is here the fallacy of the mechanical production of fibrin comes in, with the fine tubes used in the 1905 method. For in removing the end of the tube from the blood a fine thread of fibrin is sometimes seen stretching from the blood to the tube. As was shown in Section I. this may be purely mechanical and does not necessarily indicate that fibrin-ferment produced fibrin is present, but there is no way to distinguish one from the other, and the result may be that one puts a blood down as coagulated when in reality it has not.

On the whole therefore Wright's method, especially in its latest form, is unreliable, for although it is based on sound principles, in practice there are opportunities of error which it is very difficult or impossible to avoid.



looking down on lid of pot.



Bürker's Method.

2. BURKER'S METHOD

1. THE METHOD.

A sketch is given of the various parts of the apparatus, by reference to which the description will be more easy to understand.

Water at a certain temperature,(preferably 25°C) in poured into the brass pot.(1).

The temperature is maintained by a small gas burner placed under it. The lid (2) is then put on. In the middle of it a square of glass is fixed with a small concave hollow (3).

A drop of distilled water of constant size is dropped on to the slide, and covered over till it has become warmed up by the water below it.

A Francke's instrument, by which a constant depth of wound is secured, is used to obtain the blood

The first drop from the finger falls into the drop of water, and after half a minute, is mixed with it by means of five turns with a fine glass rod. At intervals of half a minute, the glass rod is drawn once to and fro through the drop, always in a different direction, until a fine thread of fibrin is seen to adhere to it. This is the end-point.

2. RESULTS WHICH BÜRKER HAD OBTAINED WITH HIS METHOD.

He gives coagulation times taken at six different temperatures and from them constructed a curve by/

by which the effect of variations in temperature might be discounted. This will be discussed in Section IV. He, also, on three successive days, took his coagulation time every 2 hours. At the time at which these experiments were done, he had not yet added to his apparatus any contrivance by which the temperature might be kept constant, and so his temperatures were those of the laboratory. These varied from 15·3°C to 21·9°C. He corrected the times of the first day by reference to his curve.

He concludes that the coagulation time is longest in the morning, diminishes until 2 o'clock in the afternoon, and then rises again. His figures are given, and his grounds for making this assertion are examined in Section IV., under the heading "Daily Variations in the coagulation time".

In connection with the elaboration of his apparatus, he made some experiments on the effect of dilution of the blood, and showed that this had little or no effect on the coagulation time.

He also made a number of experiments on the effect/

effect of varying the number of times the glass rod is drawn through the blood, showing that the oftener this is done, the more quickly does the blood coagulate, and on the effect of different sizes of the glass slide on which the blood is placed. He also showed, that there is no difference in the coagulation time of blood taken from different fingers. The coagulation time of 14 different people, taken at temperatures between 20 and 21·5° C, showed a variation of only 2 minutes. The rest of his paper deals with the connection between the Blood-platelets and coagulation.

3. CRITICISM OF BURKER'S METHOD.

I have not had much practical experience with this method, but I have used it enough to become familiar with the technique.

(A.) THE METHOD OF OBTAINING THE BLOOD.

The main objection to be taken to his method of obtaining the blood is that it takes such a long and variable time, for sufficient blood to come out to form a drop, which will fall off the finger. I noted the time which elapsed between the puncture and the fall of the drop on to the slide, in the following cases, in all of which the size of the/

the wound was the same. They were as follows.

60, 90, 75. 50. 15, 40, 50, 105, and 30 seconds.

By making the would deeper these times would, no doubt, become shorter and more constant, but the punctures I made were such, as to produce some pain, and I do not think that much deeper ones would have been practical.

The effect of contract with air of the room temperature for a time varying between 15 and 105 seconds is certainly such as will lead to a difference in the coagulation time.

Burker's method of cleansing the fingers and instruments, is also open to objection. This is especially the case in the cleaning of the glass slide on which the blood falls. This is let into a hollow in the lid, and is very difficult to clean, because some of the blood runs under the edge of the joining between the glass and the lid, and the distilled water, alcohol, and ether which he advises, only dilute it and cannot clear it away. Traces of fibrin ferment will, almost certainly, be left, and will no doubt affect the time.

(B)/

(B). THE METHOD OF MAINTAINING THE BLOOD AT A CONSTANT TEMPERATURE.

Bürker found (1) that the temperature of the blood and water, when on the slide was very nearly that of the water below it, and that differences in the temperature of the air introduced only slight differences which might be neglected.

That being granted, I yet found that it was practically impossible to keep the temperature sufficiently constant, for any length of time. The pot holds a litre of water, and the temperature he recommends is 25°C. I found that I could not keep this from varying two or three degrees, by simply turning up or down the gas jet under it. It is possible to keep it constant for the time taken by one experiment, but when you begin the next one, you almost always find that the temperature is either falling or rising, and in an attempt to alter it by regulating the size of the flame, it is very difficult to be accurate, and in any case you have to wait till it has fallen or risen to the temperature at which it was before. A thermostat is certainly required to keep such a quantity of water at 25°C.

(C)/

(1) Arch. fur die ger. Physiol. Bd. 118.

(C.) THE ADDITION OF DISTILLED WATER TO OUR BLOOD.

Bürker's main reason for adding water was that he found that without it, the first appearance of fibrin threads was very inconstant.

It is possible that the explanation of this may be that it prevents the mechanical formation of fibrin. He found that the addition of a drop of water did not have any great effect on the time when he took as his end-point, instead of a fibrin thread. the first appearance of a considerable mass of fibrin.

(D) THE AMOUNT OF CONTACT WITH FOREIGN BODIES.

The amount of contact with foreign bodies is not quite constant. The glass rod is drawn through the blood every half minute, so that when the blood takes a long time to coagulate, the rod will have passed through it many times, and when it takes a short time it will have passed through it comparatively few times. Each time the coagulation time will be diminished. This will lead, in the former case, to too short, and in the latter, to too long a coagulation time being recorded.

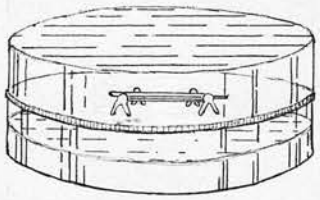
(E) THE END-POINT./

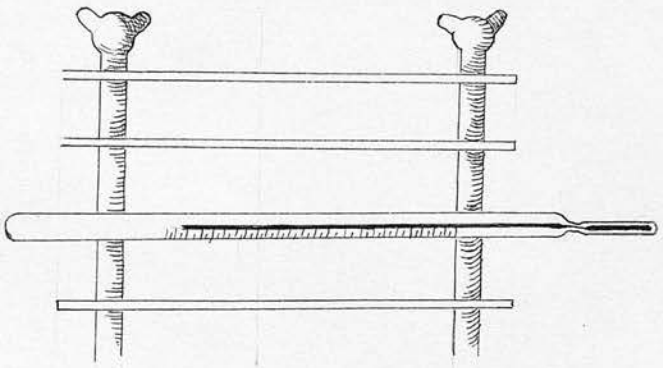
(E) THE END-POINT.

Apart from the general question as to whether fibrin threads are a good indication of coagulation I do not think that the end-point Bürker has taken is a good one. I found that, sometimes. the first fibrin picked up by the rod, was not a fine thread but a considerable mass of fibrin. In these cases the coagulation was evidently at a later stage. The rod had not happened to pick up a thread or it had been so fine that it was not seen. The time when a thread will be first picked up, must depend, to some extent also on the shape of the glass rod. Now this shape is not constant, for, to clean them from the blood, they are passed through the flame of a spirit lamp, and in the process the fine end is often slightly melted, so as to produce a little bulb at the end which would be far better adapted to picking up fibrin than one which had a smooth tapered end.

CONCLUSION.

The method of obtaining the blood is inconstant, the same temperature cannot for long be maintained, the amount of contact with foreign bodies is inconstant and the end-point is unsatisfactory.





Sabrazès' Method

3 SABRAZE'S METHOD.

Glass tubes are made with a bore of one millimetre. They are calibrated to within 2/10 of a millimetre. They are washed in an acid, an alkali, water, alcohol and ether, and dried carefully. The ends are then sealed in a flame until they are to be used when the ends are broken off. The rest of the apparatus consists of an arrangement for preserving a constant temperature of 18.5° C. Two glass boxes are taken. one being placed on the top of the other. The lower one, in summer, contains blocks of ice. In winter, the temperature is maintained by warming the air in it, by heating up the box with a spirit flame and placing a lamp near it. By moving the lamp towards, or away from the box, variations in the temperature can be cancelled.

In the upper box is a glass stand, on which the tubes, when they have been filled with blood, are placed. Here, there is also a thermometer registering half degrees centigrade.

The lobe of the ear is washed with alcohol and dried. The puncture is made with a "vaccino-style", on the blade of which, there is a mark, one millimetre from the point, or with a Francke's lancet so/

so as to get a constant depth of wound. The first drop which appears is wiped off. From the second drop, blood is allowed to enter to a height of one centimetre, in one of the tubes. The third drop is taken into a second tube in the same way. The tubes are then picked up with a pair of ivory forceps, and placed on the glass support in the upper box, as near as possible to the bulb of the thermometer. The second tube is simply taken as an indication when to test for coagulation in the first tube. It coagulates before the first. Coagulation is tested for, by seeing whether the column of blood has become so adherent to the glass, as not to move, when the tube is lightly tapped. When this is so, it will be found, that on breaking the tube across, a fine thread of fibrin will be seen, stretching between the broken ends. This is taken as the end-point. When coagulation has been found in the second tube, the first will be nearly coagulated, and the same process is gone through with it. The coagulation time in it is taken as representing the coagulation time of the blood. If the blood is immobile and yet no fibrin thread appears, it must be broken again in another place, after an interval. The first appearance of the fibrin is the real end-point.

RESULTS/

RESULTS OBTAINED WITH SABRAZE'S METHOD.

GENEUIL. (1)

He took the coagulation time of 57 patients suffering from a variety of diseases. The times varied from 4½ to 14½ minutes, including two cases of Haemophilia. In one of them it was 19 minutes and in the other 25 minutes.

No constant diminution or lengthening of the time was found in any other disease.

Thus in tuberculous conditions the time varied from 5 min. to 14½ min. In diseases of the haemopoeitic system from 8 to 12½. In malignant disease from 7½ to 13½ min. In nervous diseases from 5 to 10 min.

DISCUSSION OF SABRAZE'S METHOD.

ADVANTAGES.

The amount of contact with foreign bodies is very constant. The importance of a constant temperature is at any rate recognised, and the end-point is a clear and definite one. It is much better/

(1) Methodes pour determiner le debut de la coagulation du sang. Bordeaux 1906.

better adapted than Wright's for showing a small quantity of fibrin. When the broken ends of the tube are held up to the light and slowly drawn apart, a thread of fibrin of the most extreme tenuity can be recognised. This end-point is the same as in McGowan's Method and it will be discussed separately after the description of that method.

DISADVANTAGES.

The main defect of the method is the difficulty experienced in keeping the temperature constant.

I found in working with the apparatus that whenever the lid was taken off for the purpose of testing for coagulation the temperature fell at once to the room temperature and only very slowly rose again to 18.5° C. If the room temperature were 18.5 always it would not matter, but room temperature is never constant and in any case rooms in this country are not usually at so high a temperature as 18.5° C.

Even with all the aids Sabraze's mentions I found it impossible to keep a constant temperature. it was always fluctuating between the room temperature and 18.5, and for this reason alone the method cannot be considered to be an accurate one.

4. MC'GOWAN'S METHOD.

In this method glass tubes about one and a half millemetres in diameter, and seven inches long are used.

Variations of temperature between 15° C. and 20° C. may be neglected, at any rate for clinical purposes, so no method of maintaining a constant temperature is suggested.

The finger is slightly congested, by twisting a rag round the finger, ten seconds before puncture. After a tube has partially filled one end of the tube is sealed in a flame. After an interval of time, a file mark is made on the tube, which is then broken across. The end-point is the first appearance of a thread of fibrin, between the broken ends.

RESULTS/

RESULTS OBTAINED BY THE METHOD.

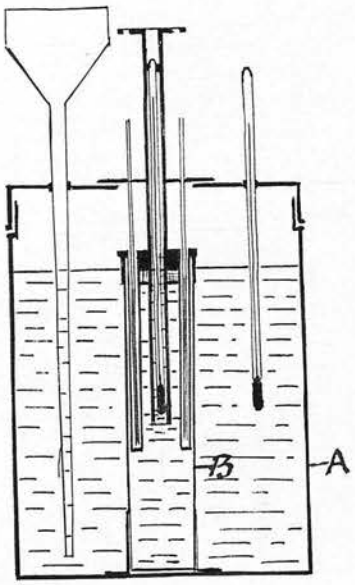
Mc'Gowan gives only a few consecutive results, to show that they do not vary much. It is evident that no accurate results could be obtained, because no provision is made to maintain an equal temperature. The following are consecutive times taken by this method, at temperatures varying between 20° C. and 15° C. They show that large variations occur, which are mainly due to the differences in temperature within these limits.

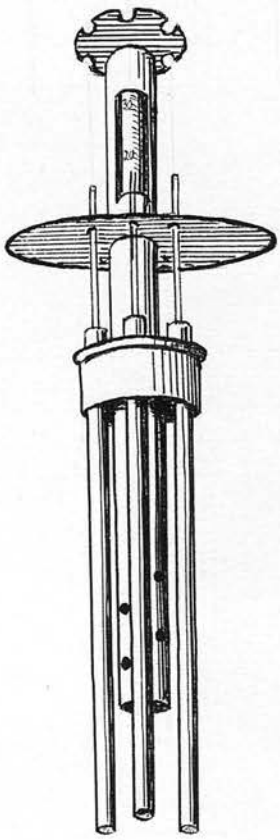
TEMPERATURE	COAG. TIMES Average of two estimations.
15·5° C	9 min 30 sec.
17·0° C	8 " 15 "
18·0° C	6 " 30 "
19·0° C	5 " 45 "

MODIFICATION/

Modification of Mc Lewis's method

Section of Apparatus





MODIFICATION OF MCGOWAN'S METHOD.

It seemed to me that if an apparatus for keeping the tubes at a constant temperature were added to this method, it would have some advantages over Sabrazès'. A drawing in section of the arrangement I adopted is given. A round tin box (A) 9 inches high and 7 inches in circumference was covered with asbestos. A thermometer, and a funnel, the end of which extends nearly to the bottom, were fixed through the lid. In the centre of this box, there is a round glass funnel which forms a second compartment. Into it, through a hole cut in the lid of the outer box, there is introduced a collection of six brass tubes closed below and open above, arranged round a central tube containing a thermometer, so constructed that though its bulb is at the centre of the inner chamber, the reading of the temperature is done outside, above the lid of the outer box.

Attached to the brass tube, which contains the inner thermometer, there is a pad of rubber, which closes the hole in the lid, through which the cluster of tubes is put. Each compartment is filled with water at 18·5° C, but only to a height at which the open ends of the brass tubes project above the level/

level of the water. The glass tubes, which contain the blood, are introduced through small tightly fitting holes, in the rubber pad, into the brass tubes. In this way air is enclosed at the top of the apparatus extends down into the brass tubes, and surrounds the glass tubes when they are in position. This air very soon remains at a constant temperature, if the water is at a constant temperature. No fresh air, or very little, can enter, because the rubber fits closely round the glass tubes. The temperature of the water is very easily regulated. It remains constant for half an hour or so, and when a fall is seen it is registered first by the thermometer in the water of the outer chamber. That in the inner chamber which immediately surrounds the brass tubes remains constant longer, because it is contained in a glass vessel surrounded on all sides by warm water. A little hot water is then poured into the funnel, and entering at the bottom diffused gradually upwards, until the outer thermometer is found to again register 18·5. In this way, with very little trouble indeed, the temperature may be kept constant for any length of time.

DISCUSSION/

DISCUSSION OF THE MODIFICATION OF MC'GOWAN'S METHOD.

In working with this apparatus considerable variations are still found. This is due to the fact that even here temperature variations are a factor in the results.

Granted that the apparatus keeps the tube at a fairly constant temperature it yet has to be taken out every half minute or so in order to test for the appearance of a fibrin thread. While it is out it is exposed not only to the temperature of the room, but also to the complicating temperature of the fingers in which it is held.

Mc'Gowan relies on the glass to prevent these variations affecting the time. but the glass is very thin, probably no thicker than the glass of the bulb of an ordinary thermometer, and yet when this is held between the fingers the temperature can be seen shooting up at once. I tried wearing rubber finger tips but they were unsatisfactory. If they were thick enough to be any good it was difficult to hold the tube.

THE END-POINT ADOPTED IN SABRAZE'S AND MC'GOWAN'S METHODS.

I wished to test the reliability of this appearance of a fibrin thread between the broken ends of a glass tube, as an indication of coagulation.

I therefore took two tubes and filled them at the same time from the same drop of blood. They were kept together so that they were subjected to the same temperature variations.

When ever a thread of fibrin was found in one tube it was immediately tried for in the other, and if none were found the attempt was repeated every quarter of a minute. This of course introduced an error because the frequent contact with the fingers must have hastened coagulation in the second tube if it had not already occurred. The difference between the two times would have been greater than it was but for this.

In order to get an average, the differences in 83 double estimations were determined.

In/

In	38	comparative	estimations	the difference was within 15 sec.
"	18	"	"	" 30 sec.
"	9	"	"	" 45 sec.
"	12	"	"	" 60 sec.
"	3	"	"	" 75 sec.
"	2	"	"	" 90 sec.
"	1	"	"	" 105 sec.

Assuming that the actual differences were at the mid-points, that is to say when the difference was within 15 sec. that the actual difference was $7\frac{1}{2}$ sec., when it was within 30 sec, that the actual difference was $22\frac{1}{2}$ sec. and so on, an average difference of 29, 27/83 sec. or practically 30 seconds is arrived at.

The average coagulation time was about $8\frac{1}{2}$ minutes, so that the percentage of difference was 5·9.

Forty five sets of double times were taken in the same way except that the tubes were filled from separate punctures both made at the same time. Rubber finger tips were used.

In/

In 14	comparative estimations	the difference was	within	15 sec.
" 8	"	"	"	30 "
" 3	"	"	"	45 "
" 9	"	"	"	60 "
" 1	"	"	"	75 "
" 2	"	"	"	90 "
" 2	"	"	"	105 "
" 6	"	"	" more than	105 "

The six double estimations in which the difference was more than 105 seconds are not taken into the calculation of the average difference as they are probably due to one of the drops of blood having been contaminated with previously formed fibrin ferment. Excluding these the average difference is 28.37/45 sec., practically the same as before.

Mc'Gowan's tubes vary somewhat in diameter. I thought it possible that the difference might be due to this.

Thirteen pairs of estimations were made with tubes, one narrow and the other wide and the average difference/

difference compared with the average difference obtained from 112 estimations in which the tubes were of equal diameter, (a single tube was broken in the middle and the coagulation times obtained in the halves compared). In both cases the average differences closely approximated to 30 seconds.

CONCLUSIONS.

Where this end-point is used to determine the fact that coagulation has taken place, thirty seconds is the average difference in the coagulation times of two specimens of blood taken at the same time and kept under apparently identical conditions.

This difference is not due to the taking of the blood, for when two punctures are used instead of one the same result is arrived at.

Neither is it due to variations in the calibre of the tubes.

The difference is probably therefore to be attributed to a defect in the end-point. That is, it sometimes happens that when blood has attained a certain degree of coagulation the appearance or non-appearance of a thread of fibrin is a matter of chance, or rather of the concurrence of certain physical/

physical agencies impossible to estimate or control.

The error however is not a large one and there is no doubt that this end-point is immensely superior in accuracy to most of the others which have been used.

2. METHODS WHICH TAKE CHANGES IN THE CONTOUR OF THE SURFACE OF THE BLOOD AS THEIR END-POINTS.

1. HEYEM'S METHOD (1)

This was one of the earliest methods evolved It consists in collecting the blood, as it issues from the wound, in flat bottomed test tubes, filling them up to a constant mark. They are then tilted at intervals, until the blood becomes so solid, that the level no longer changes with the change in position of the tube.

Hayem, however, found that his results were so variable, that he gave up the observations in which he intended to employ the method.

Dastre and Floresco(2) and Brat(3) have worked with it and the latter thinks that more accurate results can be obtained by it than with Vierordt's, or Wright's methods. Bezançon and Labbe used tubes with a diameter of seven millimeters. The blood was filled up to a height of three or four centimeters, and they were closed with a plug of cotton

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(1) Du Sang et de ses alterations anatomiques Paris, 1889 323
(2) Action coagulante des injections de gelatine sur le sang. Arch de physiol. norm et path. Par. 1896.
(3) Ueber die Einwirkung von Eiweisskorpern auf die Blutgerinnung. Berl.Klin, Wehnsehr 1902 XXXIX
(4) Traite d'hematologie Steinheil, Par. 1904 P 53

cotton wool. The same end-point was taken. This method can obviously give only very rough results, and is more of historical than practical interest.

2. MILIAN'S METHOD.(1)

Milian's method was originally intended for the study of coagulation of blood from wounds, which continued to bleed for a considerable time. The finger is cut with a lancet and the drops allowed to fall on numbered glass slides. At intervals, the slides are placed in a vertical position, and when, instead of sagging down, the drop is seen to maintain a converse contour, coagulation is supposed to have occurred and the time is noted. The following is a chart which he gives illustrating the coagulation in such a haemorrhage.

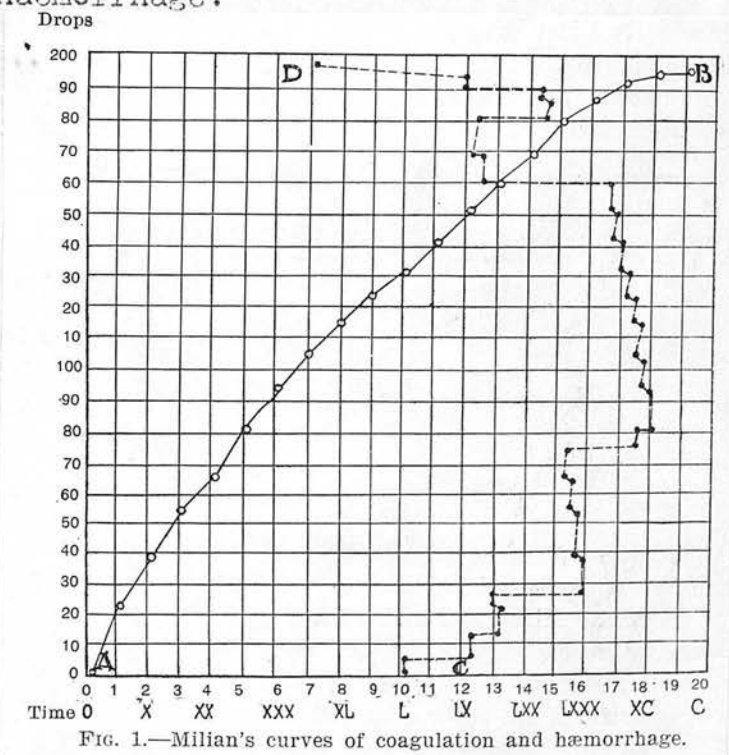

FIG. 1.—Milian's curves of coagulation and hæmorrhage.

(1) Milian. Technique pour l'etude clinique de coagulation du sang. Bull et mém. Soc. méd. d'hôp. Par. 1907.

A – B is the curve of haemorrhage and gives the number of drops which had fallen from the wound at any given time.

C D is the curve of coagulation, which shows the coagulation time at each period during the course of the haemorrhage.

Milian attributed the comparatively rapid coagulation of the last drops, which were forced out of the wound by pressure, to the admixture of fluids from the skin with the blood, and thinks that this is the most important factor; so much so that he concludes that all the methods are of doubtful utility, because they measure rather the coagulating power of the skin, than the actual coagulation time of the blood.

I have had no experience of this method but HINMAN & SLADEN (1) have worked with it and the following is their criticism: -

"In this method the recording chart has the advantage of giving considerable information about a capillary haemorrhage in a brief, concise form. This we think a very important point. The more data given, the more inferences can be drawn. As his terms imply, this is a record of much more than the simple/

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(1) John Hopkins Hosp. Bulletin. June - July 1907 .

simple coagulation time of the blood. It pictures the powers of the local tissues in their defensive reaction. Of course, the duration of the haemorrhage depends largely upon the depth of the puncture and the vascularity of the tissues. However Arthus (1) Delezenne (2), and Milian (3), have all shown that the influence of the skin is a vital factor - a most important point to bear in mind.

"For comparative laboratory methods, this might be good; but the complexity and labor involved make it impracticable for bedside and ward work. A simpler method is more desirable. In the first place it requires, as a rule, much more than the customary puncture to obtain a haemorrhage in which the blood will flow with such readiness as Milian's records show. The blood must drop from the finger. It is unusual to be able to get more than five or six drops by spontaneous flow from a wound with the lancet. From our experience, we believe an incision with a scalpel is necessary to obtain such records as 40 to 50 drops. We have found it difficult, even in this way, to obtain as many as 197 drops in what we could conscientiously/

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(1) Arthus. Compt. Rend Soc-de Biol. 1902, 93, 214.
(2) Delezenne. Compt. Rend Soc-de Biol 1897, 1898, 1900.
(3) Milian. Compt. Rend. Soc-de Biol. 1901, 576.

conscientiously call a capillary haemorrhage and Milian (1) himself states that we must confine ourselves to capillary haemorrhages alone in these practical observations."

"The second objection is that there are so many drops which must be tipped up fairly often and their profiles studied, that it is a question if two observers can work fast enough together to catch the exact moment when each one of the drops is coagulated. When the end-points begin to appear, it seems as if they all come at once. In one of our cases 28 drops reached their end-points inside of 4½ minutes. The first drop to coagulate was the last received. 22 of the 28 drops were found clotted within a period of 2 minutes - so that accurate work was impossible. The limit of error is in direct ratio to the number of drops, and must be considered in a haemorrhage such as is charted above."

"Thirdly, the times obtained are less comparable to the action of blood in a wound than in any other method. It is hard to imagine a drop of blood blood spread out to about 1 cm. diameter on a glass slide remaining fluid from 60 to 90 minutes, as some of his records show. When a drop is received by letting it fall on the slide, as in this method, it varies from ·6 to 1·25 cm. in diameter - about 1 cm. on/

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(1) Milian Presse Méd. Par. 1904.i. 202.

on the average. After coagulation has occurred, there is so much serum under the fibrin, that the contour of the drop still changes on tipping the slide vertically. By waiting until this serum evaporates sufficiently to allow the contour to remain convex on moving the slide, one can obtain "coagulation times" of 60 and 90 minutes."

BEZANÇON & LABBE'S MODIFICATION (1).

They took three drops only, on three separate slides. Their beginning point was the time when the drops fell into the slides, not the time of puncture. They do not give any details of work done with the modified method.

HINMAN AND SLADEN'S MODIFICATION (2).

Hinman and Sladen found that the size of the drop had a large influence on the time, so they modified the method, by taking drops of equal size as determined by a millimeter rule. "The 4 and 5 mm. drops are the best adapted for this method. In the smaller ones, the contour changes are difficult to judge, and the larger ones meet with the same objections as in Milian's method. The 4 mm. drop gives a definite end-point, except for very long times. In delayed times, evaporation is such an important factor, that the 5 mm. size gives more satisfactory results. The 4 mm. drop may be so nearly dried up, as to prevent judgment of an end-point. It is well, therefore, to always get a 4 and a 5 mm. drop for observation."

They give tables of coagulation times, showing that the larger the drop, the longer is the coagulation time; and also that if the drops are in a closed chamber, the coagulation is delayed, and give a comparison/

(2) Steinheil Par. 1904, p 53
John Hopkin's Hospital Bulletin June, July 1907.

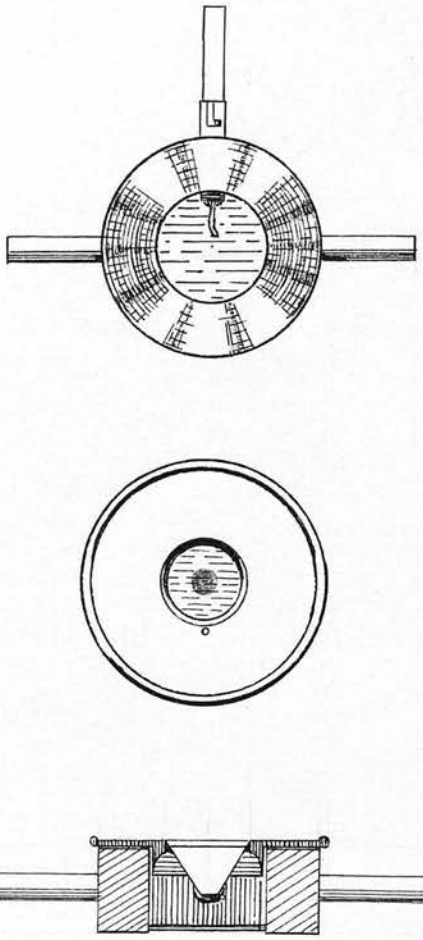
comparison of results obtained by this method, with some by Bogg's modification of Brodie and Russell's method.

They conclude - "The slide method as a quick, convenient and practical means of determining the coagulability of the blood is thoroughly reliable and can be used in the laboratory or sick room. Its simplicity is the greatest point in its favor. The fact that the whole apparatus is but a clean glass slide and a millimeter scale permits everyone to use it.

" The chief objection is the exposure and consequent evaporation. This apparently has little effect on the result in those cases where the time is within normal limits, and is no more objectionable than the blowing in the case of Brodie and Russell's method. But where the time is very much delayed, evaporation has so much better chance to modify the result that this is very often unsatisfactory. Besides the evaporation, the mechanical tilting hastens the time."

ADVANTAGES & DISADVANTAGES OF THE METHOD.

The method certainly has the advantage of great simplicity, but, besides the variability of the amount/



Bogg's Modification of Brodie and Russell's Method.

current strikes the drop of blood tangentially. A few drops of water are always kept in the box. There have been two modifications which may be conveniently described now, since the method is not essentially changed by them.

Pratt (1) discarded the truncated glass cone, and the waterjacket. A drop of blood was picked up on a cover-glass, and this was inverted in a metal box with a glass bottom, pierced by a metal tube directed as in the original method.

Boggs (2) retained the cone but did without the waterjacket. His apparatus is of better construction, and easier to work with, than Brodie & Russel's.

TECHNIQUE.

As soon as a large enough drop of blood has formed, the end of the cone is brought into contact with it. The blood will be found to flow evenly on to it, right up to the edge. Care must be taken not to dip the cone too far into the drop, so that the blood overlaps the edge.

The cone is now fitted into the box, which is placed on the microscope, and the low power focussed until the blood is seen. Then through a rubber pipe/

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(1) Pratt. Journal Med. Research, 1903, V. 120.
(2) Hinman & Sladen, John Hopkin's, Hospital Bulletin. June - July, 1907, p.213.

pipe a blast of air is driven into the box, and the drop under its influence will be seen to flow round. At first the corpuscles remain separate. Later they clump together, and the flow becomes sluggish.

Later still it will be found that the drop of blood has lost its fluidity to such an extent, that blowing on it "simply indents the rim, without causing rotation."

This is the end-point as taken by Brodie & Russell.

No directions are given as to how the circulation of water at a constant temperature is to be kept up through the water-jacket surrounding the box. This may not appear to be a difficult matter, but as a matter of fact in practise it would lead to considerable complication. A reservoir of water would have to be kept at a constant temperature with a thermostat. Some length of tubing would be necessary to carry the water to the box, when it lies in position on the stage of the microscope, so that the actual temperature of the water, when it reached the box, would vary to a certain extent with the temperature of the room. But granted that a circulation of water, at a sufficiently constant temperature, could be so attained, the temperature of the air in the/

the box certainly would not remain constant because fresh air is constantly being blown into it from outside.

As a matter of fact those who have used the method, appear to have been satisfied that variations of room temperature are of no importance.

No mention is made, as to how the air is to be blown in. Presumably, air from the mouth is to be blown through a rubber tube attached to the nozzle.

This is the method that Coleman employed. Others who have used it do not mention this point, except Hinman & Sladen who obtained the current of air by compression of a rubber bulb. This is, certainly the better way for the temperature of air blown from the mouth must vary greatly.

RESULTS/

RESULTS OBTAINED BY THE METHOD.

1. BRODIE & RUSSELL (1)

They give only a few coagulation times, illustrating the effect of temperature. It is difficult to see how they can be of any value, as the different temperatures mentioned are, presumably, those at which the water was kept, and not the temperatures of the air surrounding the blood, which is the only temperature of importance. These temperatures must have been very variable, especially when the water in the jacket was hot. In that case the temperature of the air in the box starting at a point higher than the air of the room, but considerably lower than that of the water, would begin to rise immediately the cone was fitted in. The rise, however, would not be steady, because it would vary every time air was blown in. It is obvious, that with this method, there is no chance of controlling the temperature.

2. PRATT. (2)

He used his own modification of the method.

He/

(1) Journal of Physiol.1897 XXI. 403.
(2) Journal of Med.Research, 1903 V 120.

He found that the coagulation time was extremely variable - "from 2 - 9½ minutes within half an hour in one case." "The attempt was then made to discover the factors, which caused the variations in the coagulation of blood obtained from puncture of the skin. Numerous determinations of the coagulation time, in health and in various morbid conditions, failed to yield any positive results."

3. MURPHEY & GOULD. (1)

They used Pratt's modification, and compared it with Wright's method. They conclude that Wright's method is less accurate.

Sometimes the blood cannot be got to flow at all. "This is usually due to uncleanly technique, but is sometimes caused by the exposure of the drop to a cold draught".

In 5% of the cases, no result was arrived at; while with Wright's method no result was obtained in 15%.

4. COLEMAN. (2)

Coleman preferred Brodie & Russell's method to/

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(1) Murphey & Gould. Boston Med. & Surg. Journal 1904.
(2) Bio. Chemical Journal 1907.

to Wright's, but does not discuss the method. The results he obtained by it were very consistent.

(5) HINMAN & SLADEN (1)

After comparing this method with Vierordt's Heyem's, Wright's, & Milian's methods, they came to the conclusion that it was best, "as having the greatest accuracy combined with simplicity of technique." They used Bogg's modification mainly.

After showing the difference which is produced by the variations in the diameter of the end of the cone, found in different specimens of this apparatus, they discuss the end-point. They are of opinion, that those who have used the method, have taken different stages in the process, as this end-point. Thus Pratt took the moment when the individual movement of the corpuscles ceased.

Murphey & Gould took "the moment when the edge of the drop fails to rotate in response to the air-current." Brodie & Russell considered that coagulation had occurred "as soon as a rim at the periphery is solid, and blowing simply indents this rim, without causing rotation." They describe four stages/

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(1) John Hopkin's Hospital Bulletin June-July 1907.

stages.

1. Free flowing movement of the corpuscles individually.
2. Cohesion of corpuscles & flowing movement in clumps & groups.
3. Marked elasticity, flowing having ceased; a vibratory, elastic, circular motion.
4. Elastic radial motion; indentation of the edge.

They adopt the last as their end-point, and consider that this is probably what Brodie & Russell meant.

The effect which the amount of blowing has on the time is then discussed. "The blowing on the drop, is the one great objectionable feature of the method. This increases air-contact, causes more rapid evaporation, and very likely has a considerable influence on the length of the period. If the factor were constant, the objection would be largely obviated. The observer can overcome this satisfactorily, by using very light pressure on the bulb at long intervals. Continuous blowing should not be used, and the interrupted periods of blowing should/

should be only momentary. The force of blowing can be easily judged by watching the drop."

The results which they obtained show a very variable coagulation time. In 251 determinations, 14.7 % were eleven minutes or more and 14% were four minutes or less. The limits were, from thirty three minutes to less than three minutes. Widely variable times were recorded in consecutive observations on the same person.

ADVANTAGES AND DISADVANTAGES OF THE METHOD.

One great advantage is that only one drop of blood is required. This, in itself eliminates the multiplied chances of error in obtaining the blood, which are entailed in some other methods, where several punctures have to be made, or where several drops are taken from one wound.

The method of obtaining a practically constant size of drop, is very ingenious, and simple. With a little practice, the cone can be dipped into the drop of blood, so that the blood will run right up to the edge and there is no perceptible difference in the depth of the drop so obtained. Pratt in his modification leaves this out; but it is really the best/

best point in the method.

The use of the microscope is also a great improvement. In Milian's method for instance where a somewhat analagous end-point is taken, the time at which it occurs is very indefinite and uncertain, while with Brodie and Russell's method much slighter changes are readily seen. The disadvantages of the method, however, are very important.

First of all, there is no efficient means, by which the blood may be kept at a constant temperature. This has been already explained.

Secondly the amount of evaporation is inconstant. The water at the foot of the box is intended to prevent evaporation, by keeping the air moist; but, as a matter of fact, it can have very little effect. I was never able to make out any constant difference, when the box was left dry. The inconstancy of the amount of evaporation, is due to the variability, in the amount of blowing, which different drops require. It is impossible to blow for instance once every half minute. Very variable results are obtained, if this is done. Some drops agglutinate rapidly, and if not blown on a good deal, they will give the radial indentation, which is taken/

taken as the end-point, when very little coagulation has occurred at all. That is to say, when the cone is taken out, and the drop touched with filter paper, it will be found that no clot can be demonstrated.

Thirdly, the end-point does not, necessarily, depend on coagulation at all, but may be due to simple agglutination. If a drop of blood, for instance, is set at motion, whenever it is put into the box, it will probably give a time of from 4 to 12 minutes at ordinary room temperature. If another drop be taken, under the same condition, and left undisturbed for one or two minutes, it will be found to show the end-point when it is first blown on. Even in those cases where the blood is kept moving from the first, at short intervals of time, agglutination plays an important part in the production of the end-point, for, in many cases, strong blowing is sufficient to break up the blood, from its stage of gelatinous semi-solidity, so that it once again flows smoothly round, just as it did at first.

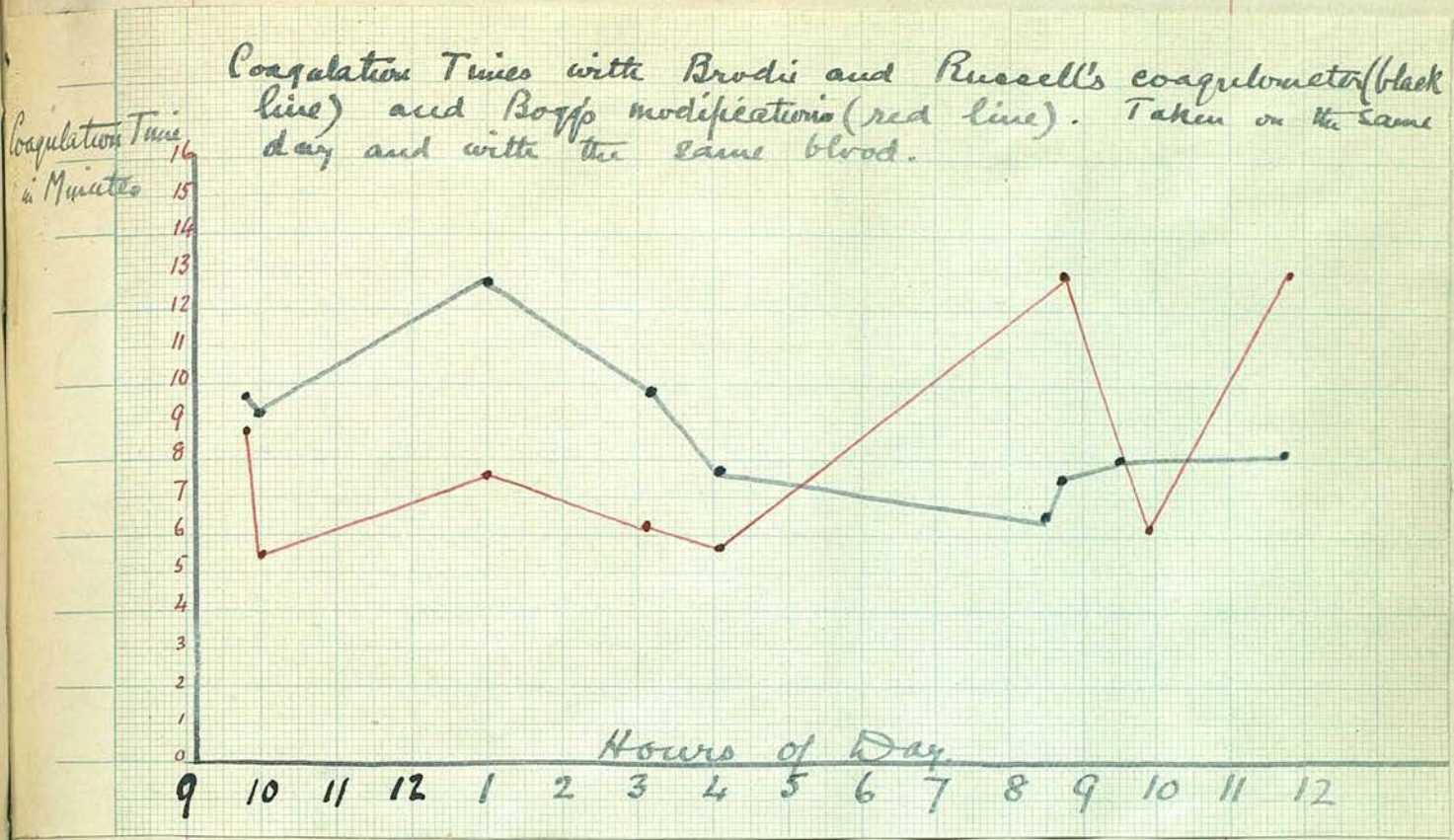
The end-point then depends very largely on the strength and frequency of the blowing, and short and long coagulation times may be obtained at will, by varying the force of the current of air.

RESULTS/

RESULTS OF PRACTICAL EXPERIENCE OF THE METHOD.

Over 200 observations were made, some with Brodie & Russell's, but most with Bogg's modification of the apparatus. These were directed to determining the effect of the variations in external conditions discussed in Section I. I then attempted to determine the effect of calcium and of the Citrates & whether there were any regular daily variations in the time. Coagulation times were also taken in a considerable number of pathological conditions. The results were discordant, and led me, ultimately, to the conclusion, that the method was unreliable.

As an example, I give a chart of consecutive observations, made with Brodie & Russell's apparatus (black); and with Bogg's modification (red) The diameter of the end of the cone was the same in both instruments, and they were taken as nearly as possible, at the same time, and under the same conditions of temperature. It will be seen that the times are very variable and that the results of the two instruments do not correspond.



3. METHODS WHICH TAKE THE NON-DIFFUSIBILITY OF BLOOD IN WATER AS THEIR END-POINT.

1 BIFFIS METHOD.(1)

I obtained the description of this method from a thesis by Geneuil(2), as I have been unable as yet, to obtain the original paper. The apparatus consists of a glass jar with a guttapercha cork. This cork is pierced by a glass rod, so inserted that it can be pushed up or down. At the end of the rod, there/

(1) Biffi. (Institut. d' hygiène de Lima) Un procedimiento sencillo para determinar la coagubilidad de la sangre La cronica medica ano XXI numero 372 Junio 1904
(2) Methodes pour determiner le debut de la coagulation du sang Bordeaux 1906

there is a platinum wire, ten centimeters long, with five loops on it, such as are used in bacteriology. They are four or five millimeters in diameter, and are separated from each other by a space of one centimeter. The jar is half filled with water at a constant temperature, and the temperature of the air in the upper half is recorded by a thermometer, introduced through the side of the jar. At fixed intervals one after the other of the loops is dipped into the water, until one is reached which instead of dissipating itself through the water, remains unchanged. This is the end-point.

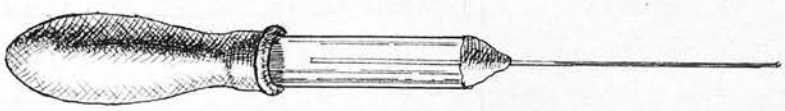
I do not know of any work by this method which has been published.

I had the apparatus constructed from the above description and tried to get results with it. I found that it was fairly easy to keep a constant temperature, provided that the jar was immersed in a basin of water the temperature of which could be kept up by adding more hot water. Thus the most important difficulty in any method was solved, but only by a modification of the apparatus. Another difficulty however, remained, that of always picking up films of equal thickness. As I shall show in discussing Buckmaster's method, the effect of evaporation is very marked in such films of blood, and different/

different results are got according to their thickness. The difference, however, though it is certainly almost always present, is seldom so marked as to be noticeable to the eye. The end-point is very gradually attained. Coagulation begins at the periphery next the wire, and works in towards the centre. The last loops show a small patch of blood in the centre, which is still diffusible.

I found when the first loop was dipped in. that water ran up the wire and mixed with the blood in the loops higher up. For this reason, I did not go on with the testing of the method, and wrote to the author, asking how he obviated this, but I have not, as yet, received an answer.

Huggston Fox's Method



2. HINGSTON FOX'S METHOD.

The apparatus consists of a series of capillary tubes, 5 cm. of which hold 5 cubic millimeters of Hg. 8 cm. long. Each is fixed into a larger tube by sealing wax. Four of the tubes are taken in succession, and nearly filled with blood, from a puncture near the end of the finger or thumb. Blood must flow freely, and the finger must not be squeezed. "Nor must the finger be ligatured, as this much lessens the coagulation time." The time of drawing the blood is noted. After the 1st tube has been filled, the finger is wiped and the puncture closed, until after an interval of 30 sec. or more, the next tube is applied and the time noted.

When some time has passed, a rubber nipple is applied and the blood in the tube expressed into water. "The process is repeated until either a definite, worm-like clot is expelled, which retains its form for some seconds, or until the contents have become so dense, that they are with difficulty expressed". "This occurs, in some cases, apart from the formation of a worm-like clot, the mass being partially diffusible in the water. The reason of this variation is, probable, the admixture of coagulable lymph.."/

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(1) Lancet January 11th 1908.

lymph".

"Should the nipple fail to expel the contents string should be wound tightly around the lower part of the nipple and then persistent efforts in compressing it, will finally succeed in emptying the tube."

"Sir A.E.Wright accounts the coagulation time to be completed from the first appearance of a shred of fibrin formation. This appearance is seen much earlier and I think may sometimes be accidental, due to the presence of a minute quantity of blood less freshly drawn, or of skin tissue, or other extraneous matter."

"It seems to me better to wait until the entire mass of blood is coagulated, as by this means we obtain a much longer coagulation period than Sir A.E.Wright's the figures are not comparable with his."

CORRECTION FOR TEMPERATURE.

Took Coagulation times at

40° F.	16 min.	On Plotting these out to scale the coagulation time is seen to form a curve, the ordinates of time increasing greatly as the temperature descends towards freezing point. to which line the curve doubtless forms an asymptote. A curve has been found which
50° F.	10¾ "	
60° F.	8½ "	
70° F.	7½ "	
80° F.	6m. 40 sec.	

closely/

closely approximates to this between the temperature of 40° F. and 80° F. but I have not yet obtained a formula for reducing observations to the standard temperature of 60° .

This correction must therefore be made from the diagram graphically."

DISCUSSION OF THE METHOD.

The method of obtaining the blood is open to great objection, and the difficulties in connection with the end-point have been mentioned by the author. There is no means to keep the temperature constant. For these reasons I did not think it necessary to do more than acquaint myself with the practical technique of the method.

4. A METHOD WHICH TAKES THE FACT THAT WHEN BLOOD HAS ATTAINED A CERTAIN DEGREE OF COAGULATION IT NO LONGER ADHERES TO A HORSE-HAIR INTRODUCED INTO IT AS ITS END-POINT.

VIERORDT'S METHOD: (1)

This method was published in 1878, and was the first clinical method.

Glass tubes with a bore of 1 mm. and 5 cm. long, are carefully cleaned. A drop of blood is obtained, care being taken to avoid dirt, pressure, and contact with foreign bodies. The temperature also must be, as far as possible, constant. Five mm. of blood are taken into the tube. Then a white horse hair, which has been previously extracted in alcohol and ether, and dried, is pulled through the tube, so that a small segment of it is surrounded by blood. At intervals of time, the hair is pulled a short distance out of the tube. As coagulation advances small masses of fibrin and red blood corpuscles will be found sticking to the hair;but later still the blood becomes so solid, that none adheres to it at all./

(1) Arch. d. Heilk. Leipz. 1878 XIX 193.

all. This is the end-point.

RESULTS OBTAINED BY VIERORDT.

He tried to determine whether there were any daily variations in the time. For this purpose, he took his coagulation time at four different periods in the day, from 11th January to 7th March.

His times varied from 3½ to 17½ minutes. No direct conclusion can be drawn from a study of the tables, but when the averages are taken the following result is obtained.

In the morning before food.	forenoon	afternoon	evening	before going to bed.
9·63	8·84	10·19	8·12	9·65.

He also made some more detailed examinations of the daily variations without, however, being able to establish any regularity in the fluctuations. He gives experiments to show that marked congestion of the finger delays the coagulation time of blood from it.

He also demonstrated experimentally that loss of a considerable quantity of blood led to an increased coagulation.

In an appendix, he gives the result of blood transfusion, in hastening the coagulation time of the blood in a greyhound. He also showed that starvation/

starvation had the same effect.

In fourteen pathological cases, including catarrhal jaundice, lobar pnumonia, and "abdominal-typhus" he examined the coagulation time. He found no marked variations though on the whole the times were shorter than in health.

His paper, although the first, is still the most complete examination of the problems connected with the coagulation time of the blood.

As regards his method, although it may compare favourably with some others devised later, still, it cannot be considered an accurate method, since it takes no precautions against variations in temperature I did not think it necessary to make any practical application of it as he himself gives such a large number of observations, by which its value may be estimated. Variations of from $3\frac{1}{2}$ to $17\frac{1}{2}$ minutes in the same individual are certainly due to experimental errors. In the coagulation times, taken on the same day, when he was determining the time at short intervals, he records variations of from $3\frac{3}{4}$ to $10\frac{3}{4}$, of $4\frac{1}{2}$ to $12\frac{1}{2}$, of 5 to $10\frac{1}{2}$ and so on. Coagulation times taken with only a short interval of time between show wide variations from $4\frac{1}{2}$ to $8\frac{1}{2}$ min. for example.

5./

5. METHODS WHICH TAKE AS THEIR END-POINT THE MOMENT WHEN FLOWING BLOOD BECOMES STATIONARY.

BUCKMASTER'S METHOD.(1)

This method depends on the fact that, when an oval loop of wire is drawn through a drop of blood, and part of the film, which is caught up in the loop, is shaken off, the corpuscles can be observed, with a lens, to flow under the influence of gravity, in response to changes in position of the loop. Thus, when it is held vertically, they can be seen to fall slowly downwards, leaving a clear circle of plasma at the upper pole; and, when it is turned upside down the reverse happens and clear plasma is left at the other pole, while, in the horizontal position, they spread themselves evenly over the loop, so that no plasma can be seen.

As time goes on, and the amount of coagulated blood increases, the flow of the blood becomes slower and more impeded, until a moment arrives at which no movement can be seen. This is taken as the end point of the method.

Buckmaster's/

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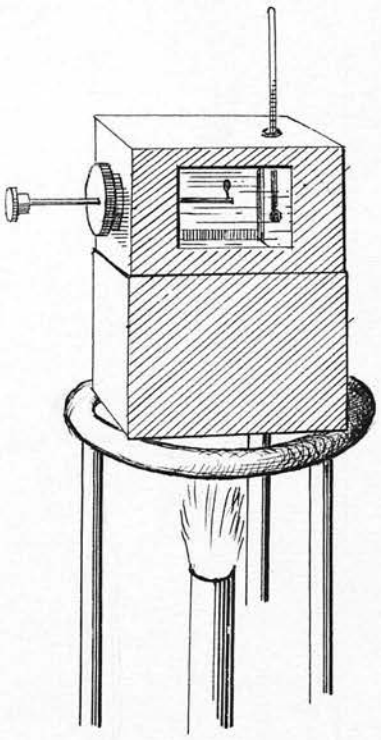
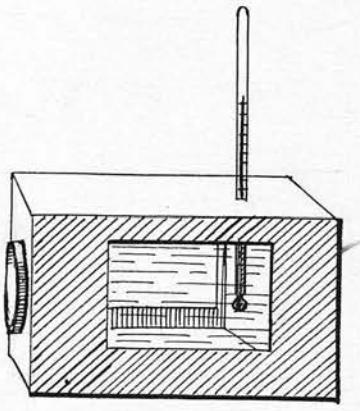
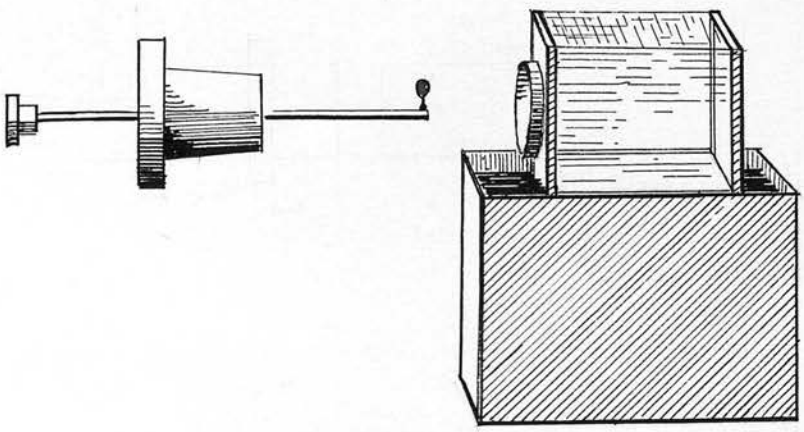
(1) Morphology of Normal & Pathological Blood 1906.







Buckmaster's Method

Buckmaster's apparatus consists of a device, by which the blood in the loop can be kept at a constant temperature. As will be seen from the illustration, which is about one third of the real size, it consists of a box, covered with felt, and with glass windows let in, on two opposite sides, The lower half is filled with water, which is heated with a spirit flame or gas jet.

Inside the box, above the level of the water, there is an inner chamber made of glass.

In the side of both boxes there is cut a hole so that a cylindrical wooden bobbin, to which the loop of wire is attached, can be inserted. When this is shoved home, the loop is situated in the middle of the inner chamber.

A thermometer pierces the roof of both chambers, so that the bulb is in the inner one, and the temperature of the air inside can be read off. The loop of wire may be of steel , platinum or silver. It can be readily cleansed in a spirit flame. It must be 5 to 6 mm. long, and 3 to 4 mm. broad. A drop of blood having been obtained, the loop is drawn through it, and a film of blood is thus picked up. It is then shaken, so that part of the blood is detached from it. This is necessary because, otherwise, the film is so thick, that it is quite opaque/

opaque, no clear zone of plasma forms on placing it vertically, and the movement of the corpuscles cannot be observed.

The bobbin is then inserted into the apparatus, and is turned so that the loop lies horizontally. At intervals of half a minute, the bobbin is rotated, so as to bring the loop into the vertical upward position, and then again into the corresponding downward position. As this is done, the cover of felt over the windows is raised, and with a lens magnifying 10 diameters the behaviour of the blood can be watched. This is done until no movement of the corpuscles is perceptible. The time is then taken, and the interval between the drawing of the blood and this time, gives the coagulation time of the blood.

Buckmaster gives only twenty or thirty coagulation times, arranged in groups which have been taken consecutively, at various temperatures. They are much more constant than those given by other methods.

PRACTICAL/

PRACTICAL EXPERIENCE OF BUCKMASTER'S METHOD. SERIES OF RESULTS SHOWING THE IMPOSSIBILITY OF MAINTAINING A CONSTANT TEMPERATURE.

The following are four series of consecutive results obtained by this method.

TEMPERATURE At beginning	TEMPERATURE At end	COAGULATION TIME. min.	COAGULATION TIME. sec.
31	31° C.	4	0
30·5	31° C.	3	30
31	31° C.	3	10
31	31° C.	3	5
35	36·5° C.	3	15
36	36·5° C.	3	15
36·5	37° C.	3	20
37	37° C.	2	30
35	36° C.	4	15.
35	35° C.	4	0
35	35° C.	4	10

TEMPERATURE At beginning		At end	COAGULATION TIME. min.	sec.
34	-	34·5° C.	5	0
33	-	34·5° C.	7	45
36	-	37° C.	3	35
37	-	38° C.	5	0
38	-	38° C.	5	15
39	-	39° C.	5	0
39	-	39° C.	3	15
38	-	39° C.	5	0
38	-	39° C.	3	30
38	-	39° C.	4	35
38	-	38·5° C.	4	15
37	-	37° C.	5	30
37	-	37·5° C.	4	30
38·5	-	39° C.	3	40
39	-	40° C.	3	50
40	-	41° C.	2	50
40·5	-	41·5° C.	3	45

It will be seen that it is only now and then that the temperature is kept constant even during the few minutes required for an experiment. This is not to be wondered at when the form of the apparatus/

apparatus is taken into consideration.

The bobbin has to be taken out in order that the loop of wire attached to it may be passed through the drop of blood. During this time the temperature of the inner chamber, being in direct communication through a large hole with the air of the room, is rapidly falling. When the bobbin is replaced it generally begins to rise again and very often is a degree higher when the blood has coagulated than when the blood was first put in.

In the first, second, and fourth of these series the attempt was made to make each observation at the same temperature so that the results could be compared.

Although every effort was made to keep the water in the lower box at a constant temperature by changing the size of the flame and by waiting until the temperature had fallen or risen to the temperature desired, the attempt was only very partially successful. So that even if the temperature variation during the time the blood is coagulating could be neglected, it would not be found practical with this apparatus to make strictly comparative estimations. Buckmaster himself does not give coagulation times taken at the same temperature. His series were taken at temperatures fluctuating very much in the same way as in the above series.

2. THE VARIABILITY OF THE COAGULATION TIMES TAKEN AT THE SAME TEMPERATURE.

In order to see what uniformity of results was attained when the temperature was constant, all the coagulation times taken were looked over and those selected for comparison which happened to be taken at the same or very nearly the same temperature. The variations while the observation was going on were disregarded, and the temperatures given refer to the commencement of the experiment.

TEMPERATURE.	COAGULATION TIMES TAKEN AT THE SAME TEMPERATURE.					
27 ° C.	7·30	5·30	7·15	7·30	9·15	6·15
27·5 "	7· 0					
28 "	5·30	9· 0				
28·5 "	7·20	6·20				
29 "	4·15	5·15	6· 0	5· 5		
29·5 "	5·15					
30 "	5· 0	8·45				
30·5 "	4·35		3·30			
31 "	3·30	4· 0	3·10	3· 5		

31·5/

TEMPERATURE.	COAGULATION TIMES TAKEN AT THE SAME TEMPERATURE.					
31·5° C.						
32 "	4·45	4· 0				
32·5 "						
33 "	7·45					
33·5 "						
34 "	5· 0	5·30	5· 0			
34·5 "						
35 "	4·15	4·45	4· 0	4·10	3·15	4·15
35·5 "	3·50	4· 0				
36 "	4· 0	3·35	4·10	3·15	3· 0	
36·5 "	3·20	4·15				
37 "	5· 0	5·15	5·30	4·30	2·30	
37·5 "						
38 "	5· 0	3·30	4·35	4·15	3·40	
38·5 "	3·40					
39 "	3·15	5· 0	3·50	4·25		
39·5 "						
40 "	2·50					
40·5 "	3·45					

From this table it well be seen that coagulation times taken at the same temperatures of ten/

ten show variations which are considerable when considered in relation to the total time.

3. CAUSES OF THIS VARIABILITY.

It is mainly due I think to differences in the amount of blood in the loop and to the indefiniteness of the end-point.

VARIATIONS IN THE AMOUNT OF BLOOD ON THE LOOP.

It will be remembered that the wire loop is passed through the drop of blood and is shaken to detach some of the blood, in order that the film may not be so thick as to be opaque.

It is this point in the technique, which is so difficult to carry out in a uniform manner, so as to leave always about the same thickness of film.

At room temperature the effect of marked differences in the thickness of the film is very great.

THIN FILM min.	sec.	THICK FILM min.	sec.	TEMPERATURE. ° C.
7	30	21	20	11·5
7	0	17	0	12·5
5	30	17	0	13·0
3	15	13	45	14·0
7	30	21	20	11·5
7	0	17	0	12·5

The/

The thinner the film the greater proportion of the blood will be in contact with air. Air probably acts as a foreign body chiefly by injuring the blood through the evaporation which it induces, so that moist air such as is present in the inner chamber of the apparatus will not have nearly so much effect as the ordinary room air to which the blood was exposed in the last experiment. I find that there are several pairs of coagulation times which happened to be taken at the same temperature, and with regard to which there is a note that in the one case the film was thick and in the other thin.

THIN FILM min.	sec.	THICK FILM min.	sec.	TEMPERATURE. C.
4	15	6	0	29
3	35	4	20	32
2	5	3	30	34
5	15	4	30	37
4	15	5	0	38

The average time with thin films is 3 min. 53 sec. and with thick films is 4 min. 40 sec. so that this is evidently one of the factors which lead to variation/

variation in the time even when the temperature is approximately constant.

6. THE INDEFINITENESS OF THE END-POINT.:

It is not always indefinite. Very often the great bulk of the blood ceases to flow almost in an instant, and the small circulation which still persists ceases within half a minute.

NOT infrequently however, part of the blood remains fluid and continues to flow although a definite clot is seen in one part of the film.

This is partly due to the fact that the clot forms in different parts of the film.

In 30 cases this point was noted.

In 13 of these coagulation extended gradually from the periphery until all the blood was stationary.

In 11 besides the peripheral clot next the wire, the central part coagulated, and blood continued for some time to circulate between the central and peripheral clots.

In 4 besides the peripheral clot longitudinal bands of coagulated blood extended from pole to pole of the loop.

In/

In 2 a crescent shaped space of circulating blood was left.

This want of uniformity in the form of the clot is an indication that the contact with the foreign bodies, platinum wire and air, is not distributed evenly all over the blood. Coagulation always commences first in the blood next the wire, for there both the wire and the air are acting. The next part is often the centre because the film is thinnest there and in proportion to the quantity of blood there is a greater amount of contact with air. When the blood in the centre is thick enough no central coagulation occurs before the rest of the film, because the gradual extension of coagulation from the periphery overtakes it, before the air has had sufficient time to produce its full effect.

If it were possible to have a film of equal thickness supported on some framework which would have no effect in producing coagulation, the air would act equally on all parts and the end-point would no doubt be an excellent one. As it is, it is not nearly such a good one as the one used in Sabrazè's and Mc'Gowan's methods.

ADVANTAGES/

ADVANTAGES AND DISADVANTAGES.

This is a very original and ingenious method.

Only one drop of blood is required, the need for ascertaining the temperature is recognised, and the part of the apparatus which touches the blood can easily be perfectly cleaned.

On the other hand it is difficult always to get the same amount of blood in the film, it is impossible to maintain a constant temperature, the foreign bodies employed act more on some parts of the film than on others and the end-point is indefinite.

CIUFFINI'S METHOD/

CIUFFINI'S METHOD. (1)

This method has just appeared, and I have included it for the sake of completeness. I have not yet got the author's paper and the following description of it is taken from the journal of the American Medical Association Vol. L No. 13 March 1908.

"Ten smooth glass test tubes, 8 cm. long with an internal diameter of 8 mm., the walls ·5 mm. thick are placed upright in a standard and 1 cc. of a ·9 per cent saline solution is placed in each tube.

One drop of blood drawn from a vein in the arm is added to the first tube, 2 drops to the second and 3 to third and so on.

Each tube is then closed with a rubber stopper and the contents mixed.

The minute when blood is added to each tube and the minute when coagulation occurs is recorded for each tube.

The normal average was found to be from 7 to 10 minutes in the tubes with from 7 to 10 drops.

In the tubes with from 3 to 6 drops the average/

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(1) Ciuffini. Medodo per lo studio delle coagulazione del sangae. Policlinico Rome. Feb. XV. Med. Sec. 1908.

average was between 12 and 25 minutes. He urges others who are studying the coagulation of the blood to adopt this technique, as the lack of uniformity in technique has led to contradictory findings in much of the research on record.

His room temperature was from 76 to 86 F."

SECTION III.

SECTION III.

A NEW METHOD OF ESTIMATING THE COATULATION TIME OF THE BLOOD.

When a stream of oil impinges against the edge of a drop of blood surrounded by oil, a streaming motion of the corpuscles is produced although the drop does not move as a whole. Under the microscope this flow will be seen to cease quite suddenly after a certain time has elapsed. This is due to the occurrence of coagulation in the drop.

The method is really a modification of Brodie and Russell's method. Instead of intermittent jets of air at an unknown and variable temperature, a continuous stream of oil at a known and constant temperature is used to produce the flow of the corpuscles. The end-point also is entirely different.

SHORT/


B
C
A
D
E
F

SHORT DESCRIPTION OF THE APPARATUS.

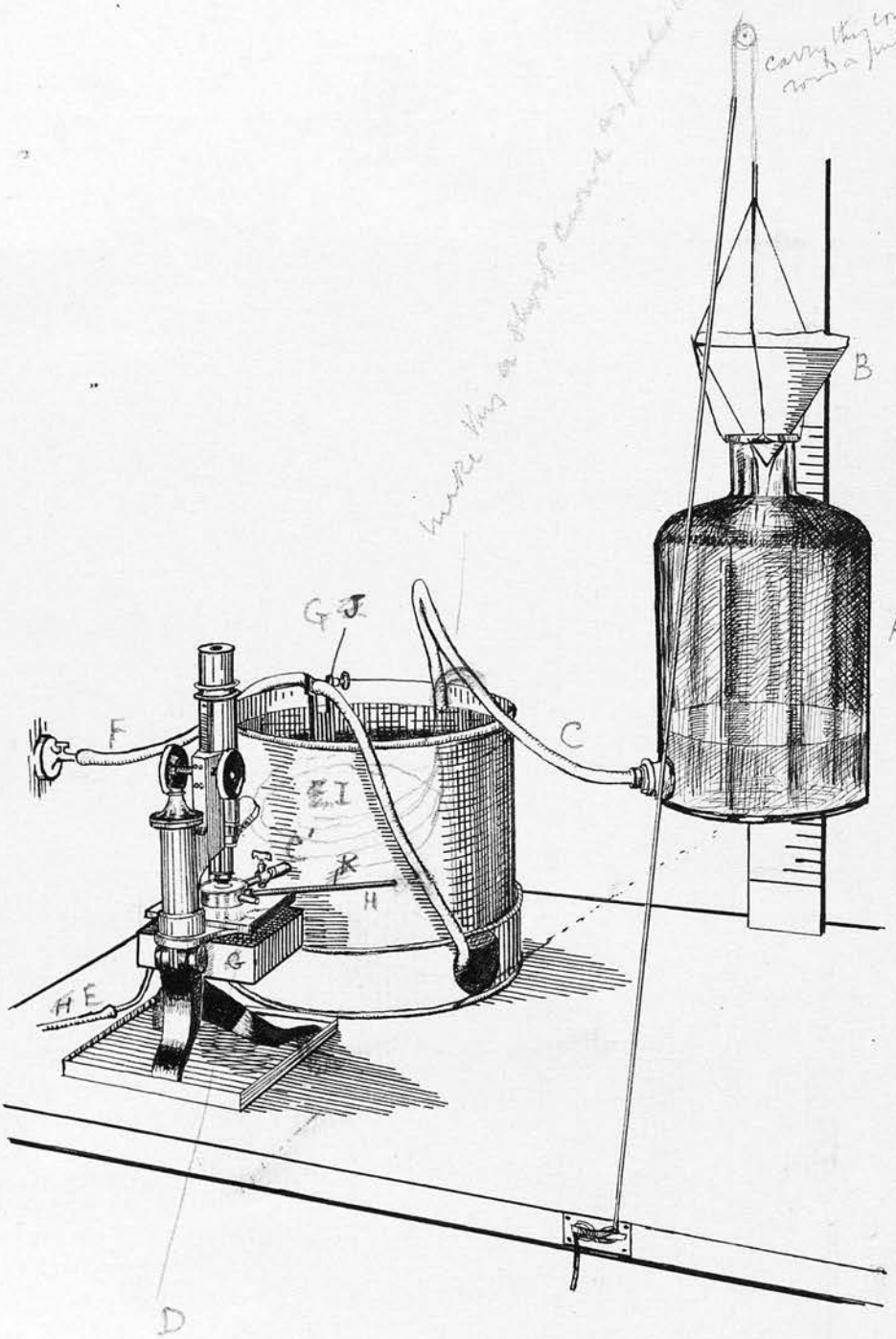
A photograph is given so that the arrangement may be more easily understood.

The apparatus is placed on a table running on rubber castors, in order that it may be wheeled up to the bedside of a patient.

A large bottle (A) containing oil hangs by a string passing through a pulley which is attached to the top of an upright (B). From this reservoir of oil a pipe (C) runs to a tank of water (D) within which it is coiled. It emerges through the side of the tank, and ends almost at once in a stop-cock.

A small tube (E) is screwed on to the outlet of the stop-cock and enters the box (F), which is Bogg's modification of Brodie and Russell's apparatus. This is placed on the stage of a microscope, which is put as close as possible to the tank. The tube ends in a fine nozzle with a bore of about half a millimeter, so directed, that the flow of oil from it impinges on the edge of the drop of blood, suspended from the end of the truncated glass cone, which forms the lid of the Bogg's coagulometer.

The oil escapes from it by a hole in the metal/


B
A
G
J
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C
I
K
H
E
D

metal fitting of the cone, and running over the stage of the microscope falls into a metal receptacle(G) suspended from it, from whence it is conveyed by a pipe (H) over the edge of the table, and filters through a funnel into a receiving vessel (I).

The water in the tank (D), is warmed by a small gas jet placed underneath it. Its temperature is regulated and kept constant by Schäfer's thermostat (J) or, where gas is not available by Professor Gibson's electrical thermostat.

The oil in the coils of the pipe (C) which are immersed in the tank, is warmed by the surrounding water. Its temperature, as it flows from the fine nozzle on to the blood, is read off on a thermometer (K).

DETAILS OF THE APPARATUS.

1. The ARRANGEMENTS for the CONTINUOUS FLOW of OIL at a CONSTANT PRESSURE.

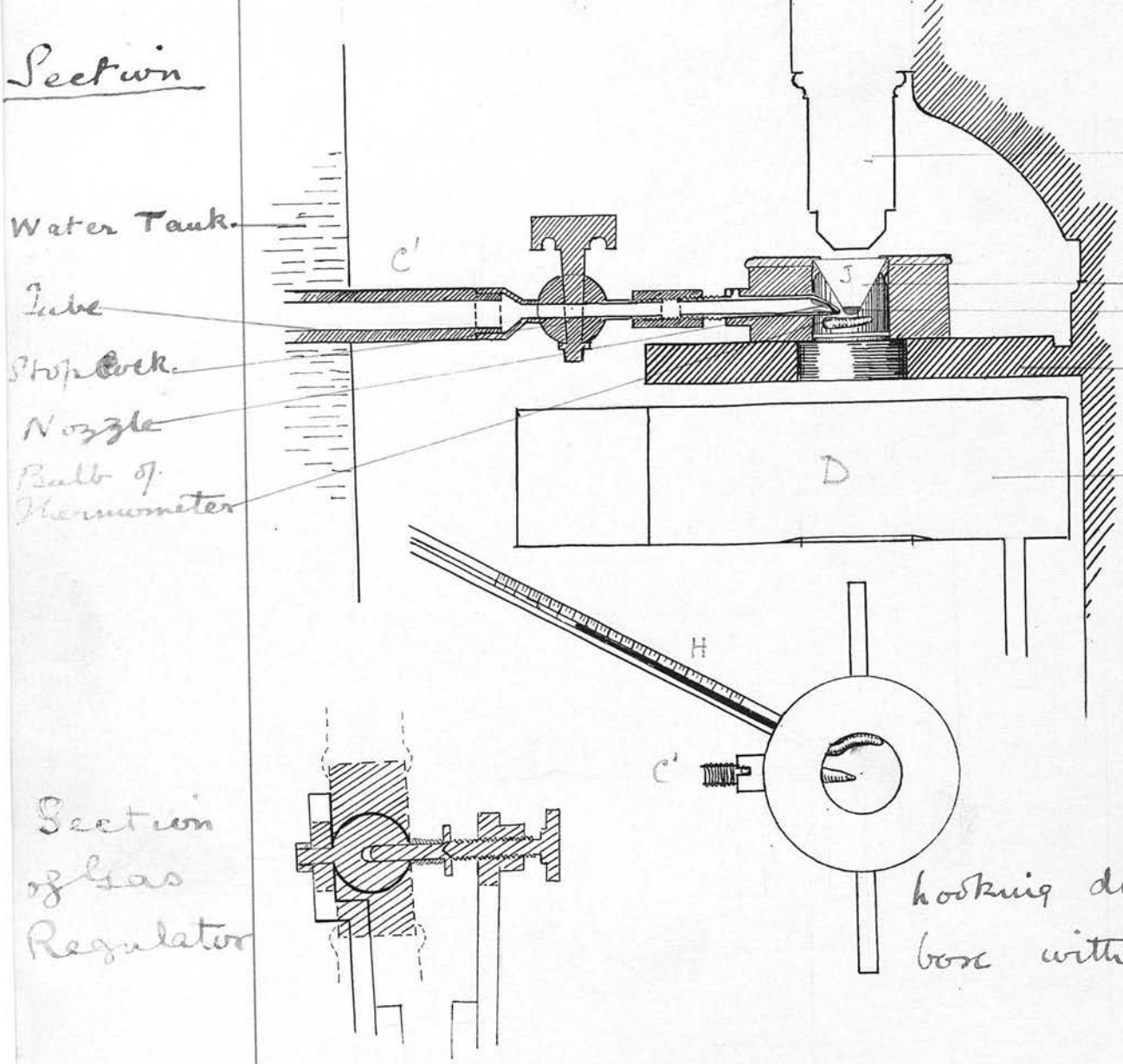
The reservoir must be such as to hold a broad surface of oil, otherwise the level falls so much during an observation, that the pressure of the stream/

stream is materially altered. A wide shallow vessel would, therefore, be best, but would have to be specially made, and the glass bottle shown in the photograph is quite efficient. It is suspended by a string passing through a pulley, in order that it may be raised or lowered.

The pressure of the stream of oil flowing from the nozzle against the drop of blood, is read in terms of the height of the level of oil in the reservoir above the nozzle. This is indicated by a millimeter scale, (not shown in photograph) attached to the upright. The oil used is the ordinary commercial paraffin which is used for burning in lamps. Oils of higher and lower specific gravity were tried. The former evaporated too quickly and the latter would not flow through the fine nozzle to be described later. Paraffin is of very constant specific gravity. It is filtered before being introduced into the reservoir. The pipe leading from the reservoir is jointed into an opening at the lowest part of the reservoir. This pipe must be flexible asbestos, lined metal tubing. It has to be flexible to allow of raising and lowering the reservoir.

It is not possible to use rubber tubing, because/

Section



Section of Gas Regulator

because oil rots rubber so quickly, and for the same reason, the ordinary flexible metal tubing cannot be used, because it is packed with rubber and soon deteriorates. A tube with a small bore imposed such a degree of resistance to the flow of oil through it, that the reservoir has to be raised to an inconvenient height. Tubing a quarter of an inch in diameter is, therefore, used.

After being coiled in the tank, it emerges through the side at the level of the stage of the microscope and ends in the stop-cock.

The metal tube, screwed on to the stop-cock, is fixed into the box which contains the glass cone with the drop of blood suspended from it, by a bayonet joint catch. To prevent this joint slipping and altering the direction of the nozzle, a brass collar is fitted on to the tube.

It has two notches cut in it which fit into pegs, one on the tube, and one projecting from the side of the box. In this way any rotatory movement of the tube in its sheath is prevented. The fine nozzle is directed at such an angle, that the flow of oil which issues from it travels across the edge of the drop, and this flow is the motive power which produces/

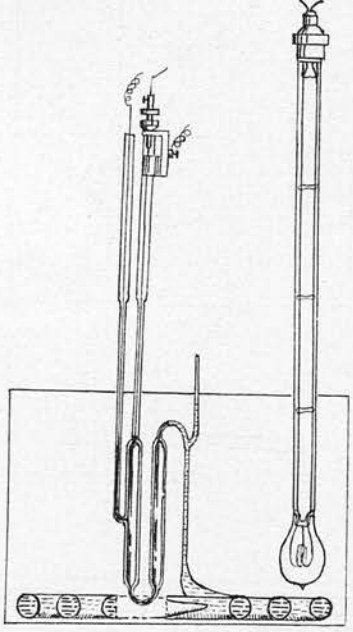
produces the rotatory flowing movement in the blood.

The nozzle is the weakest point in the apparatus. It is made of soft brass, and has to be very carefully adjusted. The slightest deviation from the proper angle leads to an irregular and intermittent action on the blood, and a great deal of trouble is required before it is brought to bear in exactly the right direction. This direction has to be determined experimentally, by moving it slightly with a pair of fine pliers, until it is found that with a low pressure of oil a smooth and uniform flow of the blood is obtained. Once the proper angle has been attained, it is best to plaster solder round it to protect it from injury, for I found on several occasions that a slight knock altered it a little, and then very many hours of work were needed, before it was put right. For this reason, I tried whether simple rotation of the whole body of the oil, would not be sufficient to produce a flow in the drop of blood, but no matter what the shape of the box nor how rapid the whirl of oil, the blood remained stationary.

I have had several boxes made in which the tube was screwed on to a fine hole drilled through its/

its side, but I have not yet succeeded in getting one, which would produce a uniform flow at a low pressure. This, however, is merely a matter of time, and when one is obtained with a drilled hole pointing in exactly the right direction, I am told that it will be possible to repeat it indefinitely, by using it as a model. The nozzle is too fine to be exactly reduplicated, but once having got it right it acts very well indeed. The box which I use at present is Bogg's modification of Brodie and Russell's, which has been described already in Section ii. The truncated glass cone, on the end of which the drop of blood hangs, forms the lid. The bottom is also of glass. The movement of the blood can, therefore, be observed by the low power of a microscope. The oil flows out of a hole in the metal fitting of the cone, and forms a little pool on the top in which the lens lies, when it is in focus. In this way the obscurity in the image of the blood which would otherwise result, through the different refractive indices of air and oil is obviated, and a very clear view is obtained.

3/



Section showing the mercurial regulator and the lamp.

2. THE ARRANGEMENT FOR THE MAINTENANCE OF A CONSTANT TEMPERATURE.

The metal pipe is 6 feet long and $4\frac{1}{2}$ feet of it are immersed in the water contained in the tank.

The tank is 7 inches high and has a diameter of 7 inches. The bottom is dome shaped, in order that the gas jet may be far enough removed from it. It is placed in a metal ring about 3 inches high, with holes for the inlet of air to the flame.

The temperature of the water in it is regulated by Schäfers thermostat.

In places where gas cannot be got, Professor Gibson's electrical regulator is used. I am very much indebted to Professor Gibson for his kindness in supervising the manufacture of a form of his apparatus which is applicable to the requirements of the method. His regulator is of the most extreme delicacy and accuracy. The details of it have not, as yet, been published by him.

With a thermostat, the water in the tank, and therefore, the temperature of the oil in the tube immersed in it, can be kept constant.

The box into which the cone fits being outside/

outside the tank, the temperature of the stream of blood flowing into it from the nozzle is usually ·25 to ·5 of a degree centigrade below the temperature of the water. The amount of variation depends on the room temperature. The temperature of the oil in the box is given by a fine thermometer reading to ·25 of a degree centigrade, the bulb of which is placed so that the jet of oil, immediately after impinging on the blood, streams on to it.

By varying the temperature of the water, by means of the regulator, the variations of the temperature of the oil in the box, which are produced by changes in the room temperature, are cancelled, and with a little trouble, a practically constant temperature can be maintained indefinitely.

THE TECHNIQUE OF THE METHOD.

I. SUMMARY OF THE TECHNIQUE.

The following points have to be attended to, in estimating the coagulation time by this method.

(1). The pressure of the flow of oil from the nozzle must be ascertained to be at the standard pressure adopted for all estimations.

(2). The temperature of the oil coming from the nozzle must be $18{\cdot}5^{\circ}$ c.

(3). The apparatus and the finger which is to be pricked, must be cleansed from traces of previously shed blood.

(4). A drop of blood must be obtained which is of a certain size and which issues immediately after the puncture. This is the starting point of the estimation, and the exact time is noted.

The glass cone must, at once, be brought into contact with the drop of blood on the finger in such a way as to pick off the same size of drop each time. This having been done, it is introduced/

introduced into the apparatus.

(5). The pressure of the oil must then be increased, by raising the reservoir to a little more than twice its original height above the blood, until a certain degree of deformity is produced in the outline of the drop.. When this has been observed, the reservoir is gently lowered until it is at its previous height.

(6). The low power of the microscope is brought into focus and the flow of the corpuscles is watched, until the end-point appears when the time is again taken.

II. DETAILS OF THE TECHNIQUE.

1. THE PRESSURE of the FLOW of OIL from the NOZZLE must be ASCERTAINED to be at the STANDARD PRESSURE ADOPTED for all ESTIMATIONS.

The pressure of the flow depends on the height of the level of the oil in the reservoir, and on the calibre of the nozzle.

With the calibre of nozzle, which is at present used the level of oil must be 10 cm. above the blood.

At one time I used a syphon arrangement for the flow of oil from the reservoir, and I sometimes found that the pressure of the out-flow of oil had altered, although the height of its level in the reservoir was unchanged. This, I think, may have been due to the shifting of air bubbles in some of the coils of the pipe, and since I gave up the syphon action, I have had very little trouble of this sort.

At the same time, the pressure may vary from the obstruction of the nozzle by dust etc. To obviate this, the oil is twice filtered, before it/

it is introduced into the reservoir.

It is, however, necessary to have a standard by which the pressure can be gauged. This is most simply attained, by measuring the length of the jet of oil when the box is removed.

When the receiving vessel , which is hung below the stage of the microscope is 3·9 cm. from the end of the nozzle, the jet of oil just falls into it. If it does not do this, there must be something wrong. The nozzle can then be screwed off and the length of the jet, as it issues from the stop-cock is measured in the same way: it should be 6·5 cm. In this way the location of the obstruction can be ascertained. This does not need to be done before every estimation, but it should be done every now and then especially if the apparatus is not being constantly used. Of course, any considerable variation in pressure reveals itself, by its different action on the blood.

2. THE TEMPERATURE of the OIL ISSUING from the NOZZLE must be 18·5° C.

This is easily brought about by regulating the temperature of the water in the tank.

Once/

Once having been attained it is advisable not to stop the flow of oil by turning the stop-cock between successive estimations, because it takes a little time for the box to be warmed up again.

The jet of oil, from its position is very little influenced by the room temperature, but it will sometimes shift ·25 of a degree above or below 18·5 with any sudden change in the temperature of the room.

This of course is rectified by the regulator; but only slowly and, in some cases, when it occurs in the middle of an observation, it may be necessary to correct it quickly by adding a little cold or hot water to the tank.

Often, however, it remains exactly at 18·5 for hours on end, without anything having to be done.

3. THE APPARATUS and the FINGER which is to be PRICKED must be CLEANSED from TRACES of PREVIOUSLY SHED BLOOD.

The parts of the apparatus which come in direct contact with the blood are the end of the glass cone and the instrument used for puncturing the skin. These, therefore, along with the finger have/

have to be freed from any possible contamination with fibrin ferment.

The only way to do this with certainty, is to expose them for some time to a temperature above 65° c. At temperatures above that point, fibrin ferment is destroyed.

The puncturing instrument, and the glass cone are placed in a vessel full of oil, the lid of which has three holes cut in it. Into one of these a thermometer is fitted and through the other two, the cone and lancet can be hung, so that they dip below the level of the oil. The vessel is placed in a tin full of water which is then heated until the thermometer indicates that the temperature of the oil is 70° c. or more. After they have been left in for a few minutes, they are fitted into the necks of small bottles containing ether. The bottles are then well shaken, and the ether dissolves off all the oil, so that when they are taken out, and allowed to dry, no trace of oil is left on them.

This is sufficient as regards the puncturing instrument, but it is of the utmost importance, that the end of the cone shall be not only free from fibrin ferment, but that it shall also be free from any/

any dust, or anything else which might impede the flow of the blood.

In the first place, to clean the drop of coagulated blood from it, it is put under a strong jet of water. Then it is washed in absolute alcohol, and put into the hot oil. After rinsing in ether, a fine silk handkerchief, free from dust, or blood, or oil, is dipped in ether, and drawn once or twice gently across the end of the cone. After that, it is fitted into the neck of a small bottle, and is thus preserved from contamination with dust, until it is required.

As regards the cleansing of the finger, although water of a temperature of 65° c. cannot be borne by most people yet when oil is used, a temperature of 70° c. is not uncomfortable. After leaving the finger for a minute or two in the hot oil, it is placed in a vessel containing absolute alcohol and afterwards a rub with a clean cloth, soaked in alcohol, will remove any remaining traces of oil. It is better to use alcohol than ether for the finger, for the latter has a greater tendency to start a fresh flow of blood from old punctures. Of course, if the finger has not been pricked before, it is not necessary/

necessary to do this. A rub with ether or alcohol, to remove the natural fat of the skin, is then all that is required.

4. A DROP of BLOOD must be OBTAINED which is of a CERTAIN SIZE and which ISSUES IMMEDIATELY after the PUNCTURE.

The following is the method which I found was best fitted to attain this result :-

A slip knot is placed round the finger, and the arm is swung round ten or twelve times. In this way the finger is filled with blood, and becomes bright red. Whenever the swinging is stopped the slip knot is tightened up, so that the condition of vascular engorgement is maintained. A superficial puncture is then made, and a spherical drop of blood about 5 mm. in diameter at once appears.

The glass cone is gradually approached to the drop. Before it has quite touched it, the blood seems to leap up and flows smoothly right up to the edge. It is put at once into the apparatus. The whole procedure from the pricking of the finger, to the fitting of the cone into its position, should not take more than 10 seconds.

Jenner's/

Jenner's vaccinostyles are very good for pricking the finger but it is necessary to fit them with a guard of some description, to prevent too deep a puncture being made. When this is done, the drop of blood is apt to spill over and a stream runs from the puncture: it is no use trying to take blood from this: the drop is always too thin. The only way to get a constant size of drop on the end of the cone, is by taking it from a drop of the right size. This is a difficulty in dealing with people whom you have not pricked before, for there is a considerable variation in the rate of flow in different individuals.

That the size of drop taken up by the cone should be approximately constant, is of considerable importance: variable results are obtained if this point is neglected. The rate of flow of the corpuscles is altered, probably because the stream of oil acts better when it impinges on a large, than on a small surface. The amount of the blood in contact with the glass of the cone, is also greater in the case of a small, than of a large drop, in proportion to the total quantity of blood.

5. THE PRESSURE OF THE OIL MUST BE INCREASED, BY RAISING THE RESERVOIR TO A LITTLE MORE THAN TWICE ITS ORIGINAL HEIGHT, UNTIL A CERTAIN DEGREE OF DEFORMITY IS PRODUCED IN THE OUTLINE OF THE DROP. WHEN THIS HAS BEEN OBSERVED, THE RESERVOIR IS SLOWLY LOWERED, UNTIL IT IS AT ITS PREVIOUS HEIGHT.

The raising of the reservoir is necessary because a higher initial pressure is required, partly to overcome the inertia of the corpuscles, but mainly I think, to break up the blood from the state of slight agglutination, into which it passes whenever it leaves the vessels.

That inertia is not the only factor is, I think, shown by the fact that the initial pressure required, varies according to the time the blood is left exposed to the action of the air. If this time is two or three minutes, it is often found that a pressure of four or five times the standard pressure, is/

is not sufficient to start the flow.

In different individuals also, there seem to be slight variations in the rate at which the blood agglutinates.

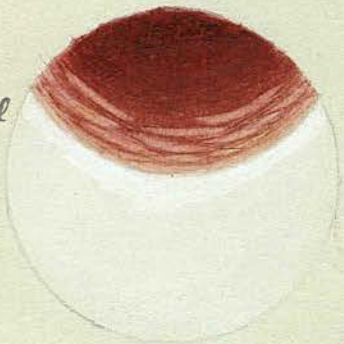
If the technique as regards the taking of the blood is strictly observed, it will be found that a height of slightly more than twice the standard pressure, will act uniformly. Thus, with my present apparatus in which the standard pressure is represented by a height of reservoir above the blood of 10 cm, an initial height of 20 to 22 cm. is required.

The outline of the drop, as the reservoir is being raised, should be watched. When the agglutination has been overcome, a tongue of blood is seen to stream out from the drop. The reservoir is, then, at once lowered; but it should be done slowly, so as to gradually lessen the rate at which the corpuscles are revolving.

6./

Blood flowing round.

Main part of blood has become stationary and a laminated clot has appeared



Total cessation of flow.

6. THE LOW POWER OF THE MISCROSCOPE IS BROUGHT INTO FOCUS, AND THE FLOW OF THE CORPUSCLES IS WATCHED UNTIL THE END-POINT APPEARS WHEN THE TIME IS AGAIN TAKEN.

When the low power is in focus, the lens lies only a few millimeters above the top of the glass cone, and is immersed in the oil which flows out there. If the necessary conditions have been observed, the corpuscles are seen streaming round fairly rapidly, each one separate from the other. The part where the flow is most rapid, is the point at which the oil stream impinges on the blood. This is the part which should be watched. After about seven minutes have elapsed without any observable change, one or two stationary streaks appear a little way from the edge of the drop. These rapidly increase in number and length, and with this there is an appreciable diminution in the rate of flow. Within a half to one minute, the streakiness will have extended right up to the edge, and will now be seen/

seen to form a laminated clot, within the meshes of which more and more corpuscles become entangled. Only a small part of total number of corpuscles continue to flow slowly and interruptedly round.

The signs of a clot associated with the cessation of flow of the great body of the corpuscles is the end-point adopted. When this is seen, the time is taken and the estimation is complete.

THE ADVANTAGES OF THE METHOD.

All the conditions which were shown in section I to be essential are fulfilled, but there are two special advantages peculiar to this method. They are (1) the certainty with which temperature variations are excluded, and (2) the close resemblance of the conditions, leading to the coagulation of the blood in the apparatus, to the conditions which determine the intravascular clotting of blood in certain pathological conditions.

I. THE CERTAINTY WITH WHICH TEMPERATURE VARIATIONS ARE EXCLUDED.

This is the only method, in which the temperature is really kept constant.

In Brodie and Russell's apparatus, air of a variable temperature is being blown at intervals on the blood. In Bürker's method it is impossible to accurately regulate the warming of the blood from below, while from above it is exposed to a variable room temperature. In Buckmaster's apparatus the heated/

heated air of the inner chamber is in direct communication, through a large opening, with the air of the room immediately before the blood is introduced into it. In Sabrazès the temperature fluctuates with each removal of the upper lid. In the other methods, the effect of temperature is either disregarded or the provision, which is made to keep it constant, is inadequate.

In my method, however, from about 10 seconds after it leaves the capillaries, to the completion of the experiment, the blood is in immediate contact with a fluid, whose temperature can be accurately measured and kept constant.

The temperature of the blood, as it comes from the capillaries, is about 37° C. In the 10 seconds or so, which are required to place it in the oil, the temperature no doubt falls and continues to fall until it reaches 18·5° C., the temperature of the oil, after which it remains constant.

As variability of temperature is the most important fallacy in other methods, so its constancy is the greatest advantage in mine.

THE/

THE FACTORS LEADING TO THE COAGULATION OF THE BLOOD IN THE VESSELS IN SOME PATHOLOGICAL CONDITIONS, ARE CLOSELY REPRODUCED IN THE METHOD.

The essential conditions of intravascular coagulation, due to injury or disease of the vessel wall, are present in the method.

In both cases, the blood is flowing. In the one case, it is surrounded by the vascular endothelium, and in the other by oil, both of which are strictly comparable to each other (1).

In the one case, coagulation is produced by friction against injured or dead tissues, and in the other by friction against glass. It is true that exposure to tissue cells has a special influence on coagulation, through the addition of thrombokinase to the blood, but thrombokinase from the puncture wound, has also been added to the drop of blood in the apparatus.

All the factors present in the one case are found/

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(1) Some have assumed that the endothelial cells secrete a substance which they term anti-coagulin. Loeb however has shown that these cells have no restraining influence on coagulation

found also in the other, so that this method gives the coagulation time of the blood, under conditions much more natural than are found in the other methods.

THE DISADVANTAGES OF THE METHOD.

The method is far from being a perfect one. This is shown by the fact that irregular variations in the time still occur even with a constant temperature.

In section IV under the heading "Daily Variations in the Coagulation Time", I have shown, that these variations are not due to alterations in the actual coagulability of the blood, but must be attributed to experimental error.

I think they are due to two causes, first slight discrepancies in the technique, and second the want of absolute definiteness in the end-point.

I. ERRORS IN TECHNIQUE.

The errors in technique most likely to arise, are those connected with the picking up of the drop of blood by the cone.

When/

When the margin of the drop of blood in the end of the cone is examined under the microscope, it will be seen that it does not always come absolutely up to the edge of the glass surface: A thin margin is sometimes left. The blood being then a very little further away, the stream of oil will not affect it in quite the same way. Again the drop is not always quite round and the flow of the corpuscles is slightly obstructed.

2. ERRORS DUE TO WANT OF DEFINITENESS IN THE END-POINT.

There are three possible stages which might be adopted as end-points; (1) the first appearance of a streak of clot, (2) the stoppage of the main flow of blood and the clear appearance of a laminated clot and (3) the complete cessation of flow.

On the average 60 seconds passes between (1) and (2) and 50 seconds between (2) and (3).

The first appearance of a streak of clot was found to be too variable to use as an end-point. In the following columns the times given by the second/

second stage, and by the third, are compared.

STAGE II.		STAGE III	
STOPPAGE OF MAIN FLOW OF BLOOD AND APPEARANCE OF A LAMINATED CLOT.		PRACTICALLY COMPLETE STOPPAGE.	
min.sec.	Variation from Mean.	min.sec.	Variation from Mean.
7·25	= -25 sec.	8·0	= -41 sec.
7·10	= -40 "	8·4	= -37 "
7·55	= + 5 "	9·10	= +29 "
7·45	= - 5 "	8·30	= -11 "
8·45	= +55 "	9·0	= +19 "
7·55	= + 5 "	8·10	= -31 "
7·55	= + 5 "	9·25	= +44 "
7·45	= - 5 "	9·5	= +24 "
8·35	= +45 "	9·5	= +24 "
8·5	= +15 "	8·45	= + 4 "
7·5	= -45 "	8·0	= -41 "
7·30	= -20 "	9·0	= +19 "

12 | 93·50 | 12 | 250 | 12 | 104·14 | 12 | 324

$7.49\frac{7}{12}$ $20\frac{10}{12}$ $8.41\frac{2}{12}$ 27

The/

The average time taking Stage II was 7min. .49$\frac{7}{12}$sec.records and taking Stage III was 8min.41$\frac{2}{12}$sec.

Counting these as 7 min 50 sec. and 8 min. 41sec.respectively, the amount of variation from these means was determined. When the second stage was taken, the average variation was 20$\frac{10}{12}$sec., and when the third stage was adopted, it was 27sec.

The second stage was, therefore, always observed as the end-point. It is the best, because it is difficult to say what the "practically" complete stoppage is. The positively complete cessation of flow is very variable, because a few clumps of corpuscles sometimes wander slowly round, for a considerable time. But the second stage, is not always quite definite, though it very often is. The main body of the blood sometimes stops in a moment. and in,the next second or two a clear laminated clot stands out. But sometimes it develops slowly, and in these cases it is impossible to be accurate to seconds, for judgment is necessary to decide, when the clot is distinctly enough visible to be counted as the end-point. The amount of possible error, however, is strictly limited, because the flow always stops completely practically within , at most, 90 seconds/

seconds after the commencement of the record stage. Now and then the main body of the blood may cease to flow without the clear appearance of any clot. This, I think, is usually due to the agglutination of the blood not having been properly overcome at the beginning. The times given in these cases, as it happens usually approximate to the mean time as nearly as most. When these slight errors in technique were noticed, or when the end-point was indefinite the resulting coagulation time has, nevertheless always been recorded, for it seemed to me that there was no advantage in making the technique too difficult and laborious. All the coagulations given, therefore, are consecutive observations, except in a few cases where, through accident, a gross error arose.

CONCLUSION.

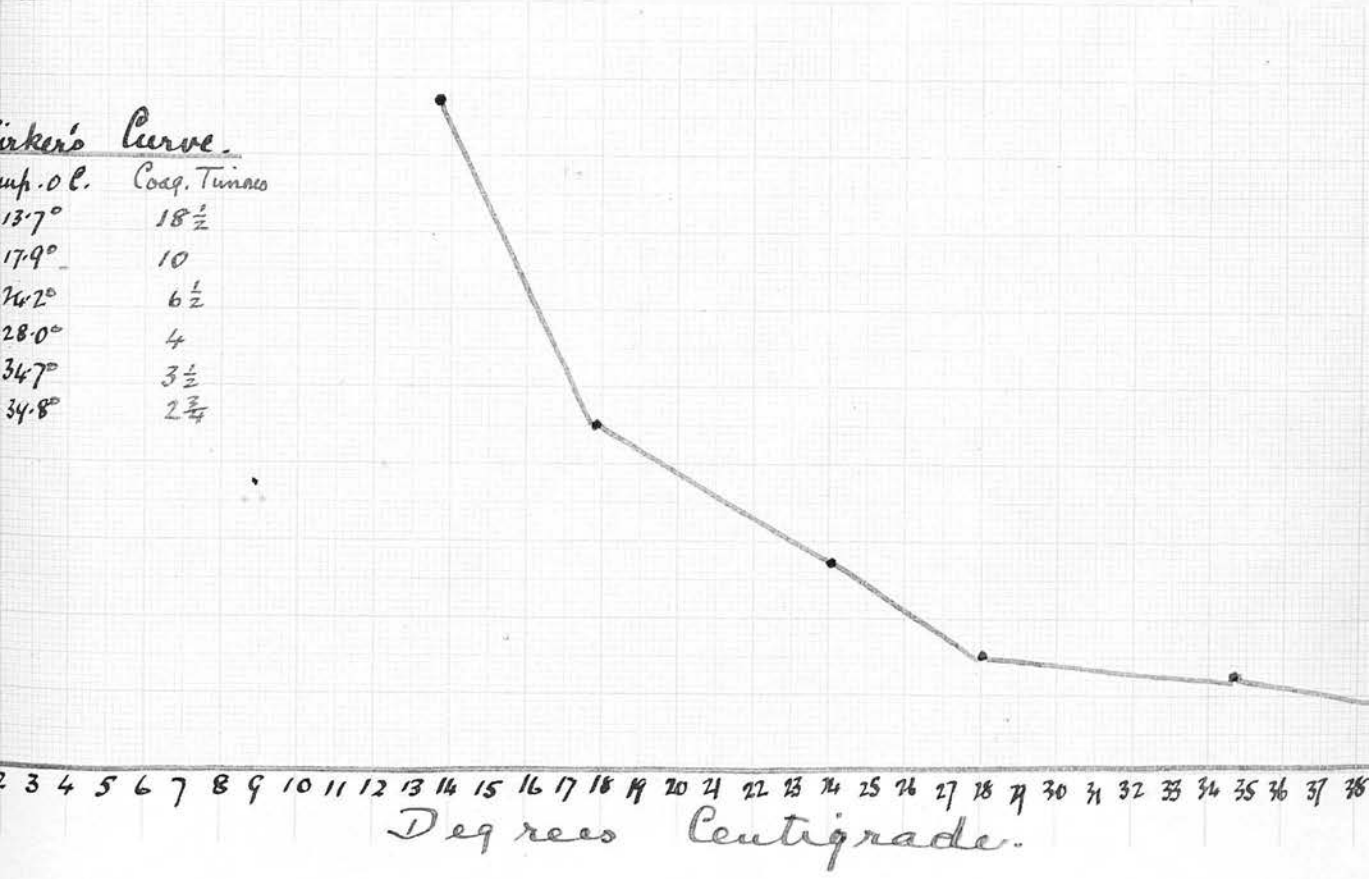
Theoretically the method is a good one, and in spite of the liability to experimental error, the results which follow from its use are more correct than any which have been obtained before.

SECTION IV.

I. THE EFFECT OF TEMPERATURE ON THE COAGULATION TIME.

Brodie & Russell (1) were the first to apply the coagulometer to the study of the effect of temperature on the coagulation time.

Bürker (2) used his method for this purpose and the following is the curve he constructed from his observations.



(1) Brodie & Russell. Journal of Physiology 1897 XXI 403.

(2) Bürker. Pflüger 's Archiv. Bd. 102 S. 67. 1904.

Hingston Fox (1) also gives some times illustrating this point. Brodie & Russell's results have been discussed in connection with their method. Bürker's curve is complicated by the fact, that the different temperatures given refer to the temperature of the water lying below the drop of blood, and do not correspond to the real temperature of the blood itself which is in contact with the air at ordinary room temperature in each observation.

Hingston Fox's results probably come nearer than either of the above to the true effect of temperature, but the results in this method are complicated by the method of obtaining the blood and by the want of precision in the end-point.

The following results obtained by my method are not absolutely true indications of the influence of the different temperatures for there is the exposure of the blood to room temperature while it is being placed in the oil. This exposure only lasts for about 10 seconds, so that it cannot have very much effect, and after that the effect is uncomplicated, and there is no doubt as to what temperature the blood is being kept at.

Temperature/

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(1) Hingston Fox. Lancet January 11th, 1908.

TEMPERATURE °C.	COAGULATION TIMES.	
	min.	sec.
3.25	63	20
7.25	32	45
10.25	21	30
12.25	16	30
13.5	14	32½
14.5	12	58½
15.5	11	46½
16.5	10.	10
17.5	8	27
18.5	7	34½
19.5	6	2½
20.5	5	22½
24.0	5	0
26.0	3	40
28.0	2	55
30.0	2	35
32.0	2	15
34.0	1	40
36.0	1	25
38.0	1	30
40.5	1	30
42.5	1	40
44.5	2	25
45.5	2	55
46.3	3	30
47.25	3	10
48.25	3	30
49.5	3	30
51.5	5	15
53.5	?	

Below 10°C. the blood showed a tendency to become agglutinated and twice the ordinary pressure was necessary to keep it flowing. The end-points were quite typical.

Above 40°C. the same phenomenon occurred.

At/

At temperatures of 53°C. and above agglutination became so marked as to interfere with the flow of the blood. At 53.5°C. there was no evidence of clot after 6 minutes but the stream was very slow and irregular. At 56°C. the blood refused to flow at all but collected in whorls and clumps of agglutinated corpuscles. On reference to the curve which has been constructed from these figures it will be noticed that the effect of temperature is not a regular one. At temperatures from 3.25 to 12.25 the diminution in the coagulation time for each degree of temperature becomes rapidly less. Thus from 3.25°c to 7.25°C., a fall of 4°C., there is a diminution of the time of over 30 minutes while from 7.25 to 12.25 a difference of 5°C., the diminution is only 16½ minutes. From 12.25 downwards there is a diminution of about 2 minutes for each degree of temperature. This amount of diminution gradually diminishes till about 28°C. it is only about half a minute for each degree. From 28°C. to 36°C. the diminution is much slower but still progressive. At 36°C. the shortest coagulation time of all is obtained. Above 36°C. the coagulation time very slowly begins to lengthen up to 56°C at which temperature the heat coagulation of fibrinogin prevented any fibrin-ferment coagulation.

The fact that there is this gradual lengthening of the coagulation time at temperatures above the normal temperature of the body is very interesting. So far as I know it has not been shown before. The amount of increase is very small, but it shows at any rate that fever is not a predisposing cause of thrombosis.

The effect of temperature as shown in this chart is also a strong argument in favour of the fibrin-ferment theory of coagulation. Nolf(1)has recently brought forward a theory in which he denies the existence of fibrin-ferment and looks upon coagulation as a mutual precipitation of two colloidal substances. If this were so, temperature would not have nearly so great an effect on the time, whereas it has just the effect which might be expected if coagulation is due to a ferment.

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(1). (Nolf.) Arch. intern. de physiol. vol. III. I.1904.
~~(2)~~ Arch. intern, de physiol. Vol. IV. 2. 1906.

DAILY VARIATIONS IN THE COAGULATION TIME.

II. DAILY VARIATIONS IN THE COAGULATION TIME.

There is no such thing as a daily variation in the coagulation time.

The results of previous work on this subject will first be reviewed and an endeavour made to explain the contradictory results obtained by different observers, before the results on which the above statement is made, are given.

A. CONCLUSIONS ARRIVED AT BY THE USE OF OTHER METHODS.

A. VIERORDT. (1)

He made a large number of observations on this point. His results were very variable, but no regularity in variability was was found. The averages of all the coagulation times, taken at different periods of the twenty four hours, were about the same. They were as follows.-

In the morning before food.	Fore-noon.	After-noon.	Even-ing.	Before going to bed.
min.	min.	min.	min.	min.
9.63	8.84	10.19	8.12	9.65

B/

(1) Arch. d.Heilk. Leipz. 1878 XIX 193.

B. Bürker (1).

Bürker took his coagulation time every two hours on three successive days. From the results he obtained he concludes that there is a regular daily variation in the time. He states that it is longest in the morning, diminishes until it reaches its lowest point about 2 o'clock p.m., after which it rises again. On going over his results it is difficult to see how he was able to draw any deductions from them at all. It is true that the times taken on the first day happened to follow the order he gives, but the results on the other two days do not. This is not apparent at the first glance because when these times were obtained he had not yet added to his method the apparatus which is intended to keep a constant temperature. They were therefore taken at a varying room temperature. The temperature is noted for each coagulation time and he corrects the results of the first day by reference to his curve, subtracting them from what, according to the curve, they would have been at that particular temperature. The results on the other two days he does not correct in this way/

(1) Bürker. Pfluger's Archiv.Bd. 102 S.67 1904.

way and of course it is only when this is done that they become comparable at all. The following three tables are the coagulation times he gives. The figures printed in red are the only ones which are comparable. They show that the variations were in reality entirely different in the time of their occurrence, so that he is not justified from his results in drawing any conclusion except that the daily variations are irregular.

7th August.

TIME	TEMP.°C.	COAG.TIME according to curve of effect of temperature.	ACTUAL COAG. TIME.	DIFFERENCE.
6a.m.	16 .9	12 minutes	14½ minutes	2½ minutes
8a.m.	17.1	11½ "	9½ "	- 2 "
10a.m.	18.6	9½ "	8 "	- 1½ "
12	19.4	9 "	7 "	- 2 "
2p.m.	19.3	9 "	5 "	- 4 "
4p.m.	19.8	8½ "	5½ "	- 3 "
6p.m.	19.7	8½ "	6 "	- 2½ "
8p.m.	19.3	9 "	7 "	- 2 "
10p.m.	19.2	9 "	7½ "	- 1½ "

8th August.

TIME	TEMP.°C.	COAG.TIME. according to curve of effect of temperature.	ACTUAL COAG. TIME.	DIFFERENCE.
6a.m.	15.3	14½minutes	12minutes	-2½ minutes.
8a.m.	16.6	11½ "	8 "	-3 "
10a.m.	17.9	9½ "	8 "	-1½ "
12a.m.	18.6	9 "	7½ "	-1½ "
2p.m.	19.6	8½ "	6½ "	-2 "
4p.m.	20.3	7½ "	7 "	-½ "
9.45p.m.	19.6	8½ "	5½ "	-3 "
11.40p.m.	19.6	8½ "	6 "	-2½ "

9th August.

TIME	TEMP.°C.	COAG.TIME. according to curve of effect of temperature.	ACTUAL COAG. TIME.	DIFFERENCE.
6a.m.	17.8	10 minutes	11 minutes	1 minute
8a.m.	18.9	9½ "	8½ "	- ¾ "
10a.m.	19.6	8¾ "	7 "	-1¾ "
12	20.3	8½ "	5 "	-3½ "
2p.m.	21.9	7 "	4½ "	-2½ "
4p.m.	21.3	7¼ "	5½ "	-1¾ "
6p.m.	21.1	7¼ "	5½ "	-2½ "
8p.m.	21.4	7½ "	5½ "	-2¼ "
10p.m.	21.6	7½ "	5½ "	-2¼ "

c/

C. Coleman. (1).

He used Brodie and Russell's method and came to exactly the opposite conclusion from Bürker. He gives the following table as a typical example of a large series of experiments.

8 am.	Coagulation Time =	2 min. 30 sec.
9 am.	Breakfast.	
11 am.	Coagulation Time =	3 min. 10 sec.
11 - 1·30	Walking	
1·30 pm.	Dinner	
2·30 pm.	Coagulation Time =	5 min. 30 sec.
3· 5 pm.	Walking	
5·30 pm.	One cup of tea	
7·30 pm.	Coagulation Time =	3 min. 50 sec.
8·15 pm.	Supper	
10·,0 pm.	Coagulation Time =	4 min. 30 sec.

The coagulation time is shortest therefore in the morning, and longest in the early hours of the afternoon.

(1) Coleman. Bio-Chemical Journal. Vol. II. No. 4 1907.

D. Hinman and Sladen. (1)

Using Bogg's modification of Brodie and Russell's method they come to no definite conclusion because they have not enough results to base one on, but they are inclined to think that the time is longest in the morning.

They give the following chart on which are seen two curves, one taken with Brodie and Russell's method and the other with their modification of Milian's method. These were made on the same day each working separately from the other.

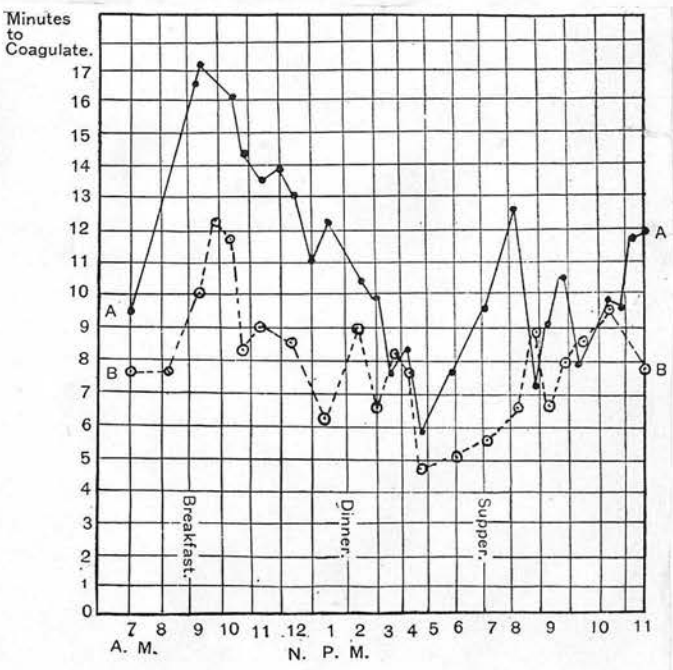


FIG. 2.—Daily curves of A and B.

(1) Hinman and Sladen. John Hopkins Hospital Bulletin. June - July 1907.

2. SUMMARY OF THE CONCLUSIONS ARRIVED AT BY THE USE OF THE OTHER METHODS.

Vierordt's results point to there being a very large but also a very irregular variability in the coagulation time.

Bürker's times show a variability not so great as Vierordt's but one which is also irregular.

Coleman's chart, shows that the shortest time is in the morning and the longest in the afternoon.

Hinman and Sladen using the same method as Coleman, though not laying down any definite rule, come to the exactly opposite conclusion. No general conclusion can be drawn from the work of these observers, for their results contradict one another.

3. THE CAUSE OF THIS WANT OF UNIFORMITY.

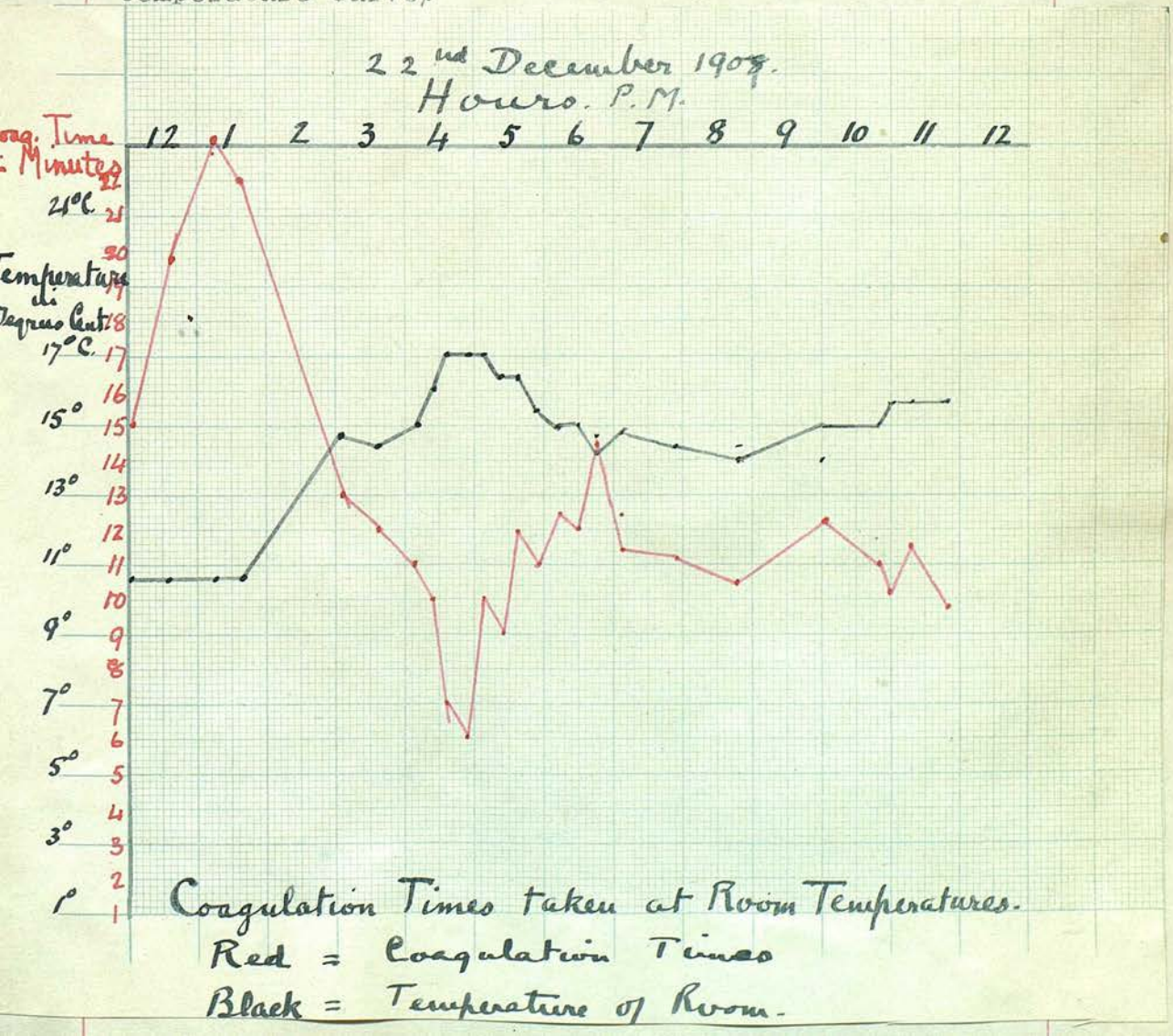
The following is an illustration of how easy it is to arrive at an erroneous conclusion in this connection.

Before I had perfected my method I attempted to determine whether daily variations occurred by using/

using Mc'Gowan's method.

On seven successive days I took my coagulation time nearly every hour. Two observations were made each time and the average result put down as the coagulation time. In this way experimental error was lessened.

On the first day I used Mc'Gowan's method exactly as he published it. The temperature at which each coagulation time was taken was noted down. The following is the chart which was thus constructed, the red being the coagulation curve and the black the temperature curve/

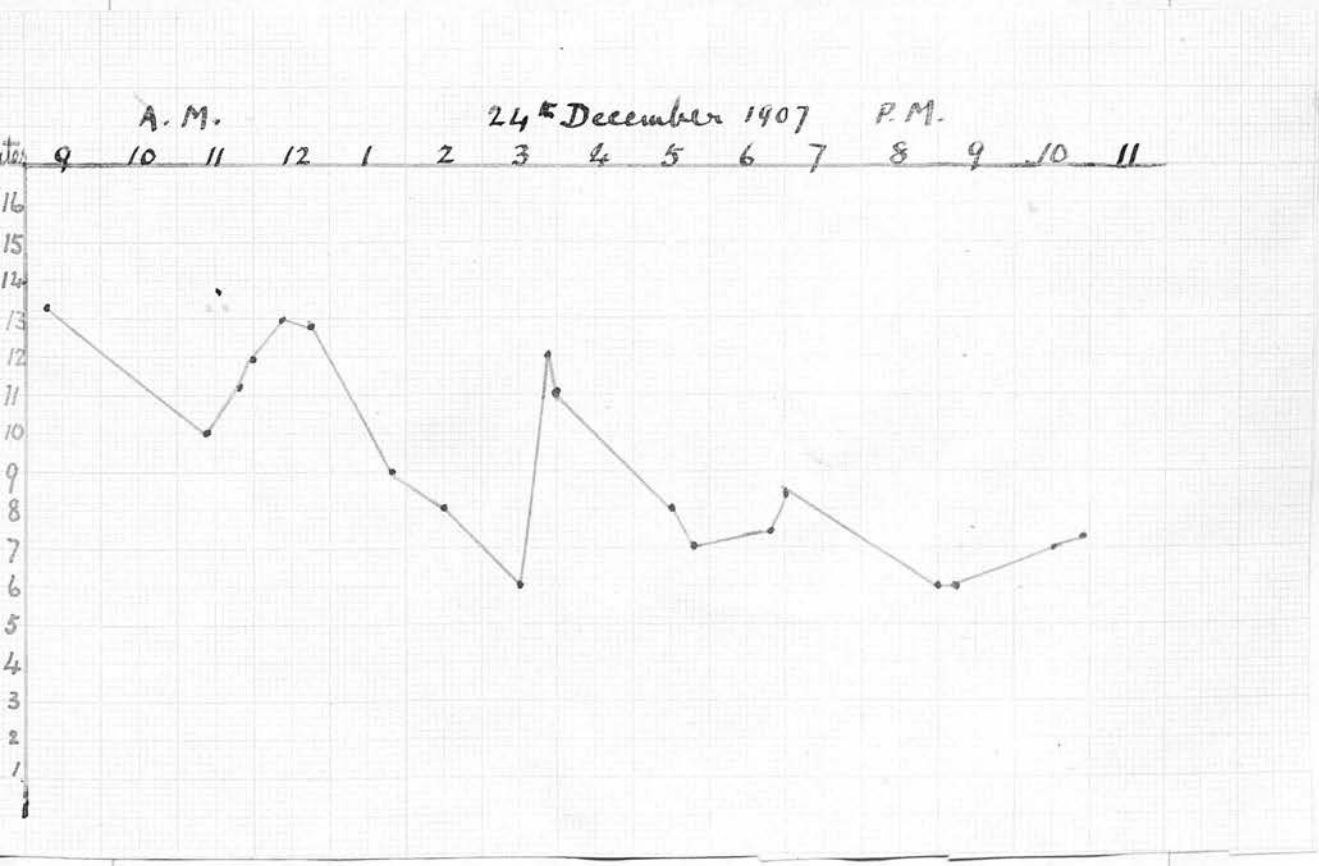


curve.

It was then noticed that the coagulation curve ran in an opposite direction to the temperature curve, so that variations of room temperature were evidently not negligable.

I therefore modified the method by placing the tubes close to a thermometer which was situated in a part of the room where it registered a temperature of 15·5. On the next four days I obtained charts of which the following is an example.

The/

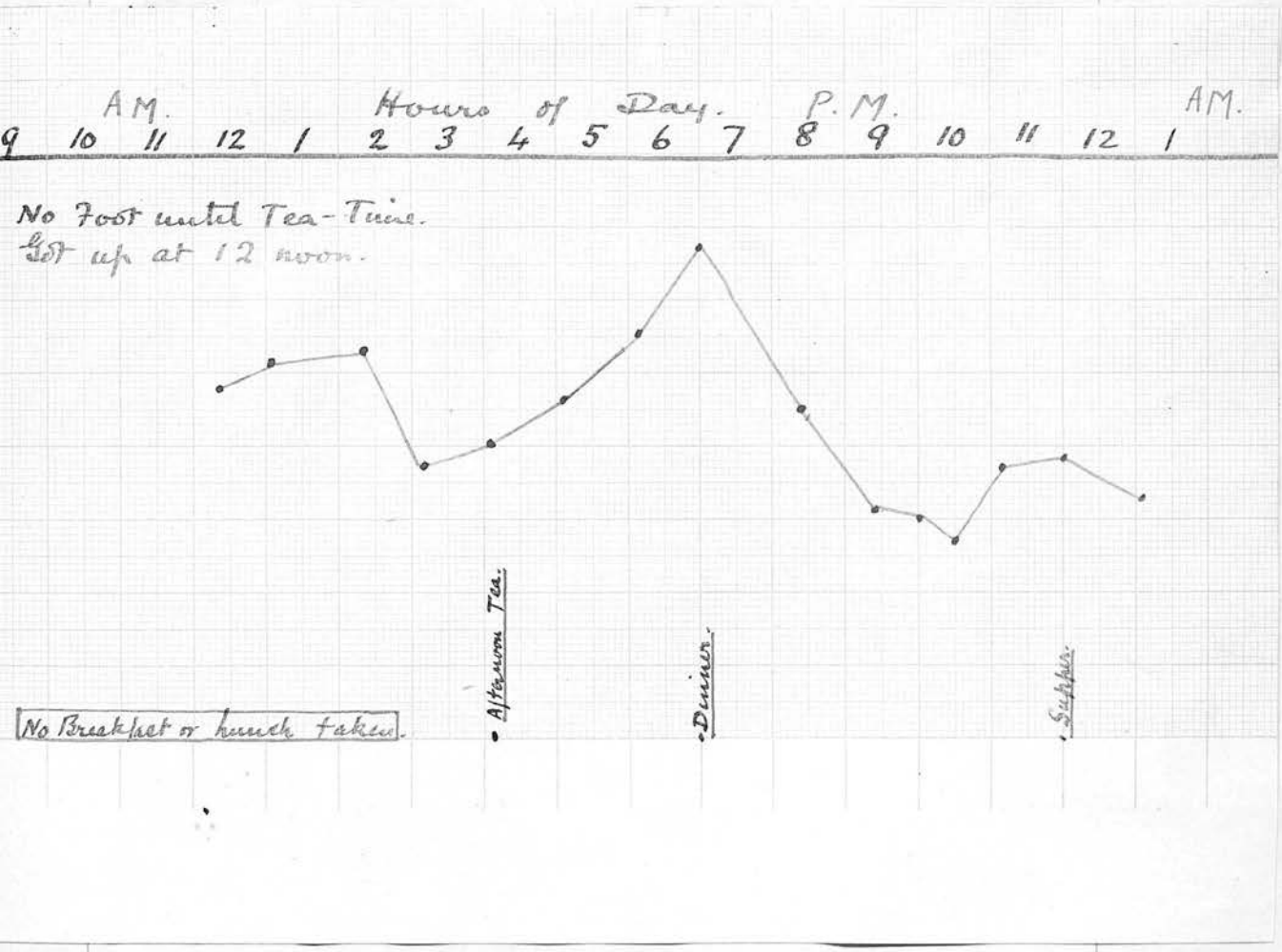


The variations were not so great though they were still considerable. They occurred at no regular time. The only constant thing about them was that the coagulation time which was taken before breakfast was always 13 or 14 minutes.

It then struck me that possibly the variations were due to the absorption of food and that the constantly delayed coagulation which I obtained before breakfast was due to the fact that at that time no food absorption was going on.

Next/

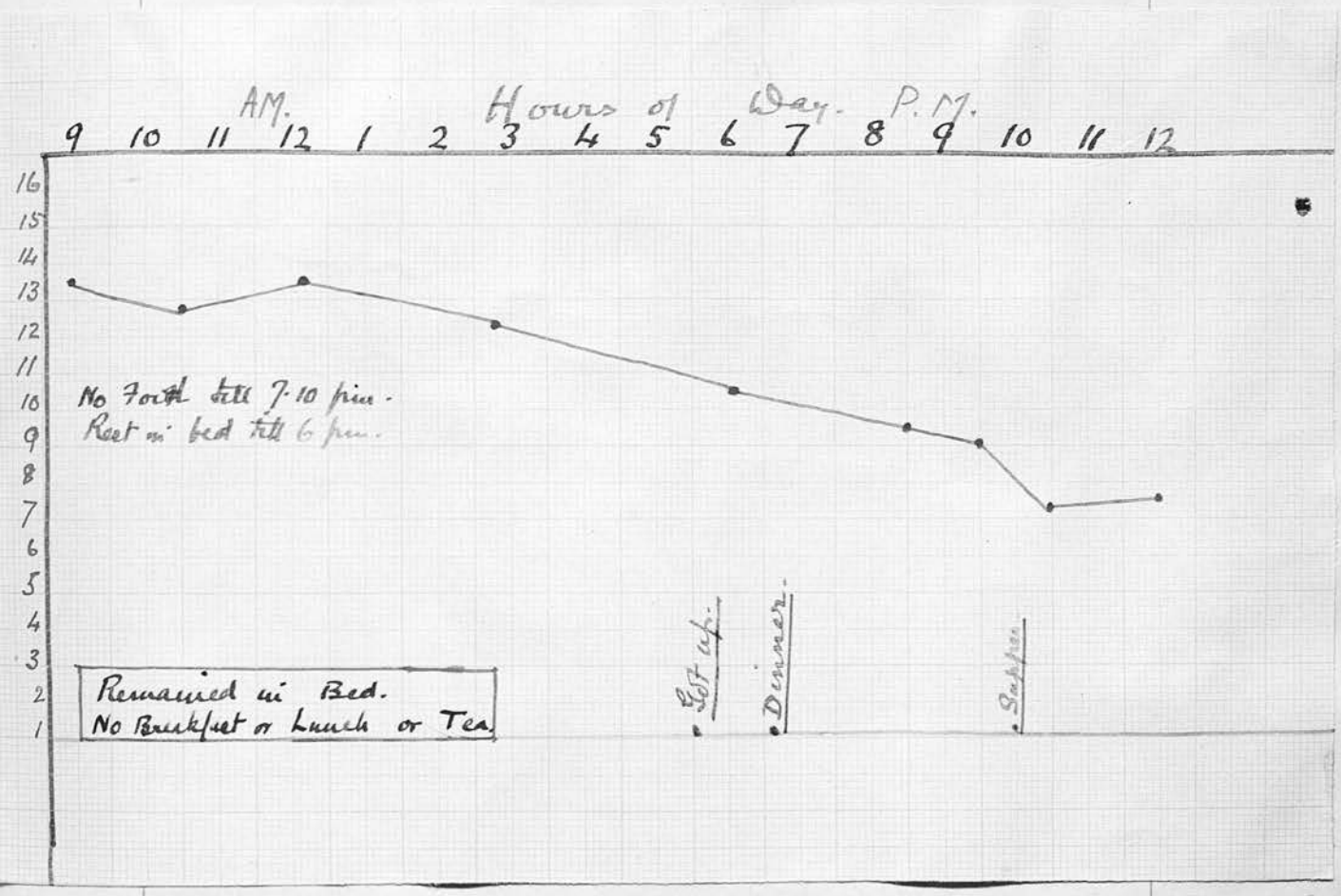
Next day I took no food until tea-time.
The following is the chart recorded.



This was not very conclusive but I thought this might be due to the complicating effect of exercise.

On/

On the next day therefore I remained in bed the whole day, only getting up now and then for a few minutes to take my coagulation time. The following is the chart.



This shows a constantly long coagulation time while I remained without food and in bed.

I now thought that I had found out the cause of/

of the great variability in the coagulation time, for I imagined that I had excluded the effect of temperature. I proceeded to confirm or disprove these results by observations on patients in the Royal Infirmary. In the meantime however I had constructed the apparatus described in Section II. under "A modification of Mc'Gowan's Method," and I found that when I used it there was no lengthening of the coagulation time in the patients in the morning before they had had food or exercise. This was not due to pathological conditions for two of them had practically nothing wrong with them.

I did not understand this until after I had worked with my method for some time, when I found that there was no such thing as a daily variation in the coagulation time and that it remained constant at all hours.

The explanation then occurred to me and I think it is one which will also explain the contradictory and confusing results obtained by others.

When I came in to the room before breakfast, the fire had just been lit and the temperature of the greater part of the room was very low (probably about 10° C.). I had to place the thermometer on a chair close/

close to the fire before it registered 15·5° C. The tubes when filled with blood were placed beside it, but every half minute they were taken up away from the fire in order that fibrin threads might be looked for. During this time they were exposed to a temperature considerably below 15·5° C. and for this reason a longer coagulation time was found than after breakfast, when all the room had been warmed up.

The constantly delayed coagulation time which I obtained when I abstained from food and remained in bed, was due to the fact that the room on that day never warmed up because the fire was allowed to remain low until the evening when I got up.

This explanation applies to the results obtained by other methods. The variations are due to the effect of temperature variations. Vierordt's results were obtained at varying room temperatures.

The want of consistency in Burker's corrected results is due to the fact that in his method there is a considerable experimental error, besides that which is due to variations in temperature.

Coleman's results are more difficult to explain. His times are much shorter than those obtained by others with the method. He does not state at what/

what temperature they were taken.

The chart given by Hinman and Sladen resembles pretty closely the first of the charts shown which were compiled from results obtained with Mc'Gowan's method. They must be regarded as an indication of the temperature of the room rather than of the coagulability of the blood.

B. THE COAGULATION TIME IS CONSTANT AT ALL TIMES OF THE DAY.

Over 300 coagulation times taken with my method were arranged according to the hour in which they were done. The averages of the sums of those between 8 am. and 6 pm. are given. fractions have been missed out.

From 8 to 9 am.	Average Coagulation Time	=	7 min. 36 sec.
" 9 to 10 am.	" "	=	7 min. 50 sec.
" 10 to 11 am.	" "	=	7 min. 24 sec.
" 11 to 12 noon	" "	=	7 min. 37 sec.
" 12 to 1 pm.	" "	=	7 min. 46 sec.
" 1 to 2 pm.	" "	=	7 min. 28 sec.
" 2 to 3 pm.	" "	=	7 min. 49 sec.
" 3 to 4 pm.	" "	=	7 min. 41 sec.
" 4 to 5 pm.	" "	=	7 min. 50 sec.
" 5 to 6 pm.	" "	=	7 min. 54 sec.

These averages show that there is no constant difference.

In the following charts the details are seen in graphic form.

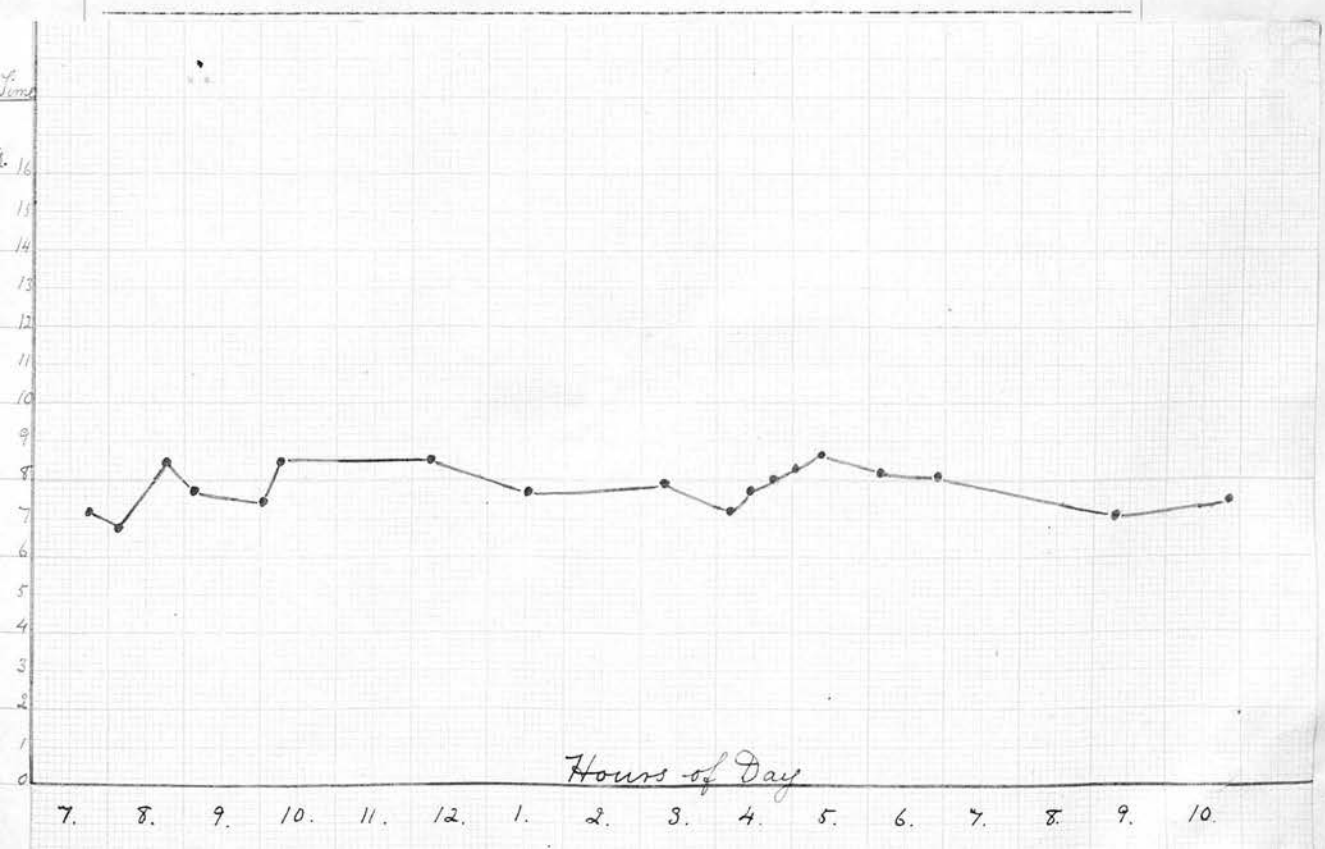
The/

The variations which are seen are due not to real changes in the coagulation time of the blood but to the experimental error inherent in the method. the causes of which have been discussed in Section III.

CHARTS SHOWING THAT THE COAGULATION TIME IS CONSTANT AT ALL HOURS WITHIN THE LIMITS OF EXPERIMENTAL ERROR.

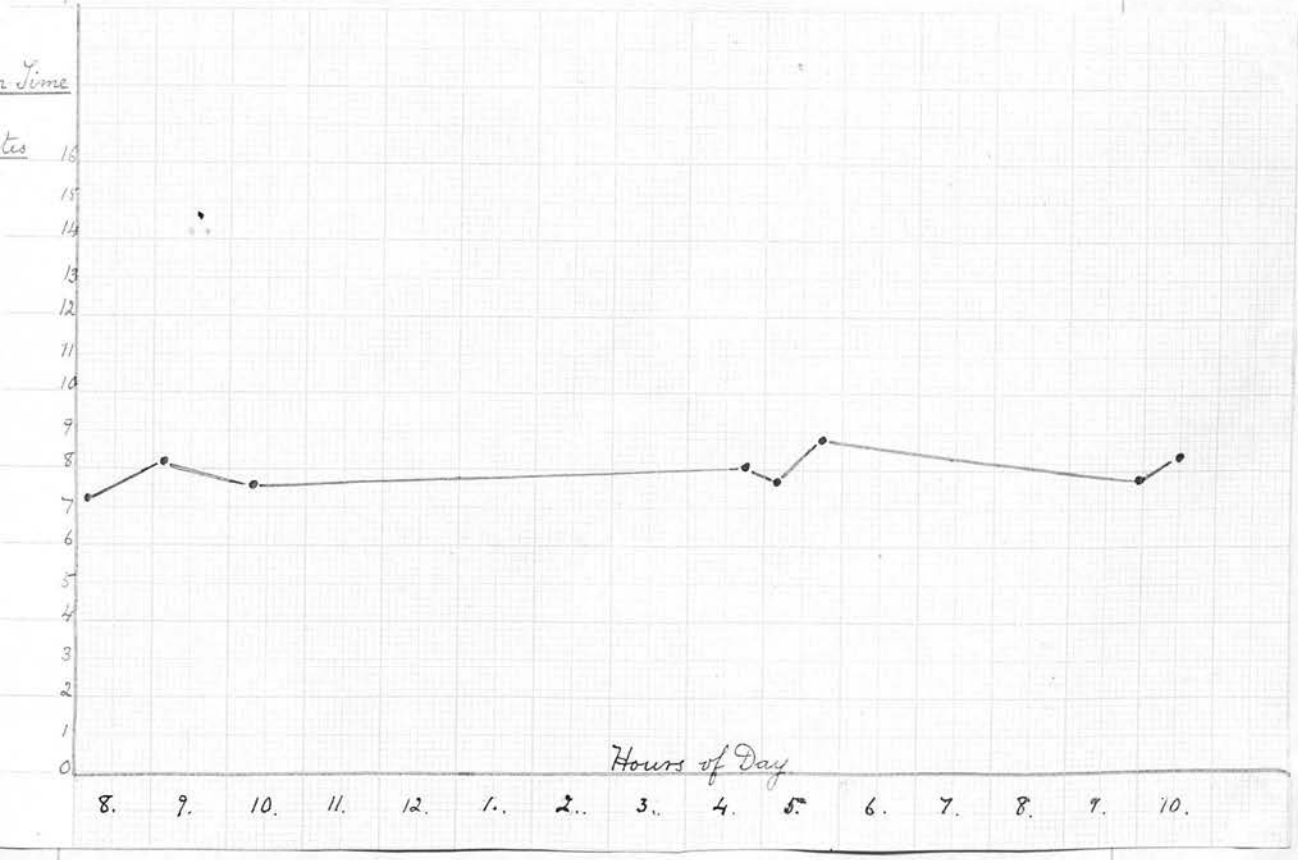
T.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
7	44	50	=	7	10
8	8	35	=	6	50
8	47	15	=	8	30
9	8	30	=	7	45
10	2	0	=	7	30
10	16	30	=	8	30
12	18	0	=	8	30
1	31	50	=	7	40
3	19	35	=	7	55
4	11	0	=	7	15
4	25	15	=	7	45
4	47	0	=	8	0
5	4	30	=	8	15
5	23	55	=	8	35
6	8	30	=	8	10
6	54	30	=	8	5
9	20	0	=	7	5
10	50	10	=	7	30



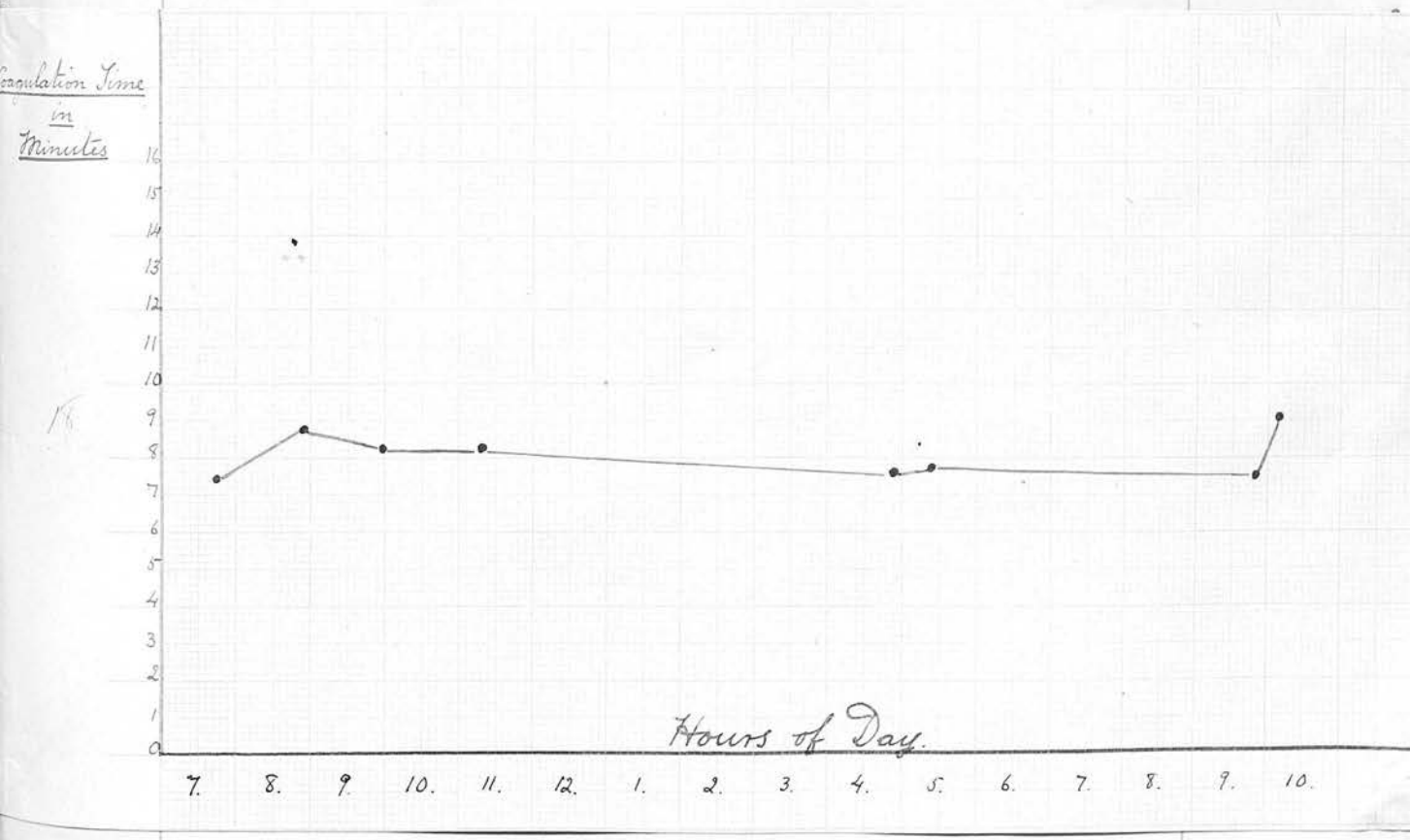
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
8	8	35	=	7	10
9	10	35	=	8	10
10	18	55	=	7	35
4	41	0	=	8	5
5	9	35	=	7	40
5	46	40	=	8	45
9	57	15	=	7	50
10	30	45	=	8	25



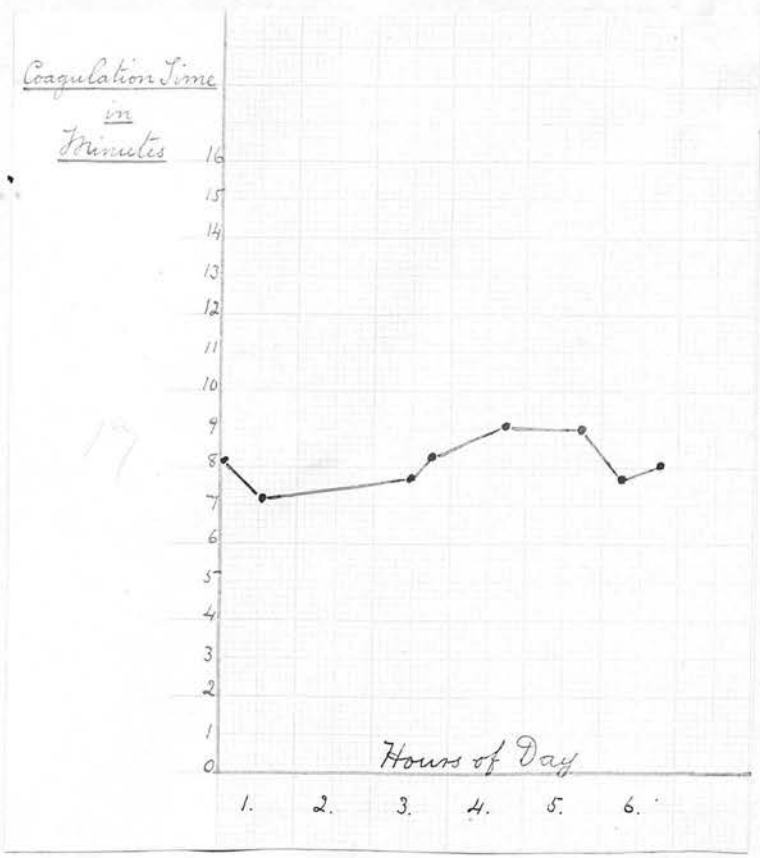
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
7	44	50	=	7	25
8	53	45	=	8	45
9	57	35	=	8	15
11	23	0	=	8	15
4	54	40	=	7	35
5	24	0	=	7	45
9	48	45	=	7	30
10	10	15	=	9	5



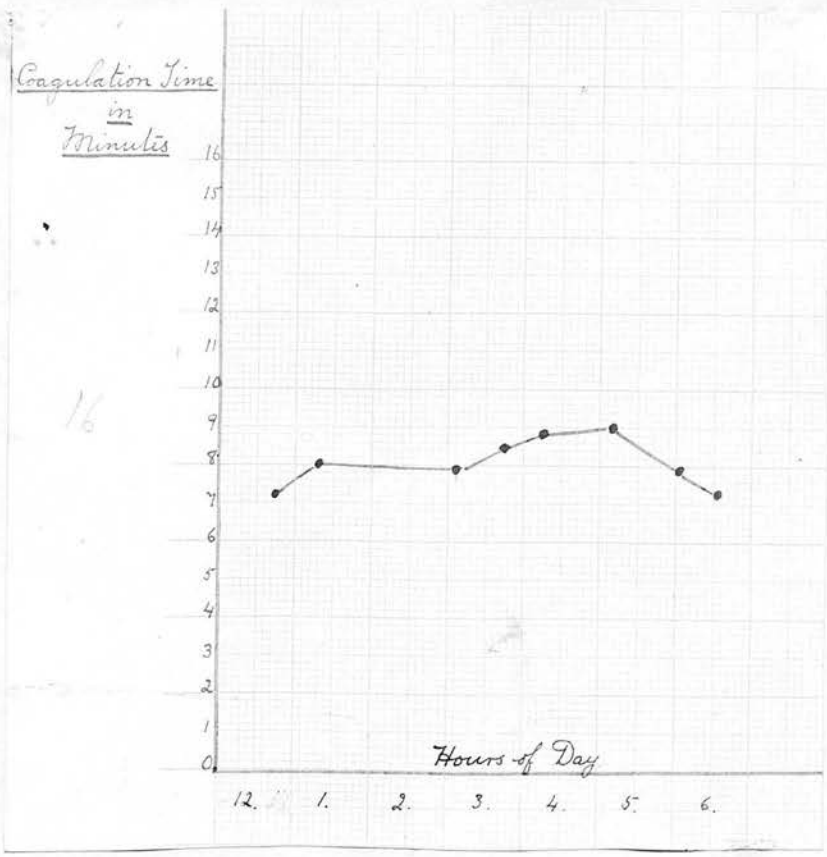
D. Mc.R.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
1	4	30	=	8	10
1	34	20	=	7	10
3	28	15	=	7	40
3	48	5	=	8	15
4	41	25	=	9	5
5	45	30	=	9	0
6	16	0	=	7	40
6	47	10	=	8	5



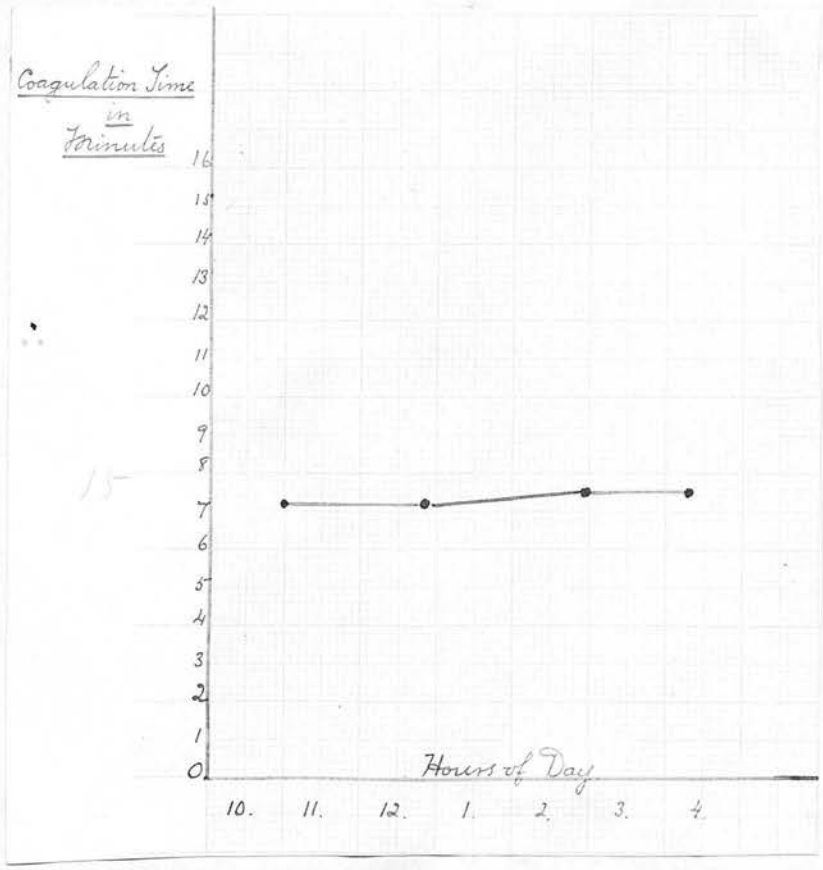
D. Mc.R.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
12	47	45	=	7	15
1	20	0	=	8	0
3	6	35	=	7	55
3	43	8	=	8	27
4	16	8	=	8	52
5	9	10	=	9	0
6	2	35	=	7	50
6	32	18	=	7	18



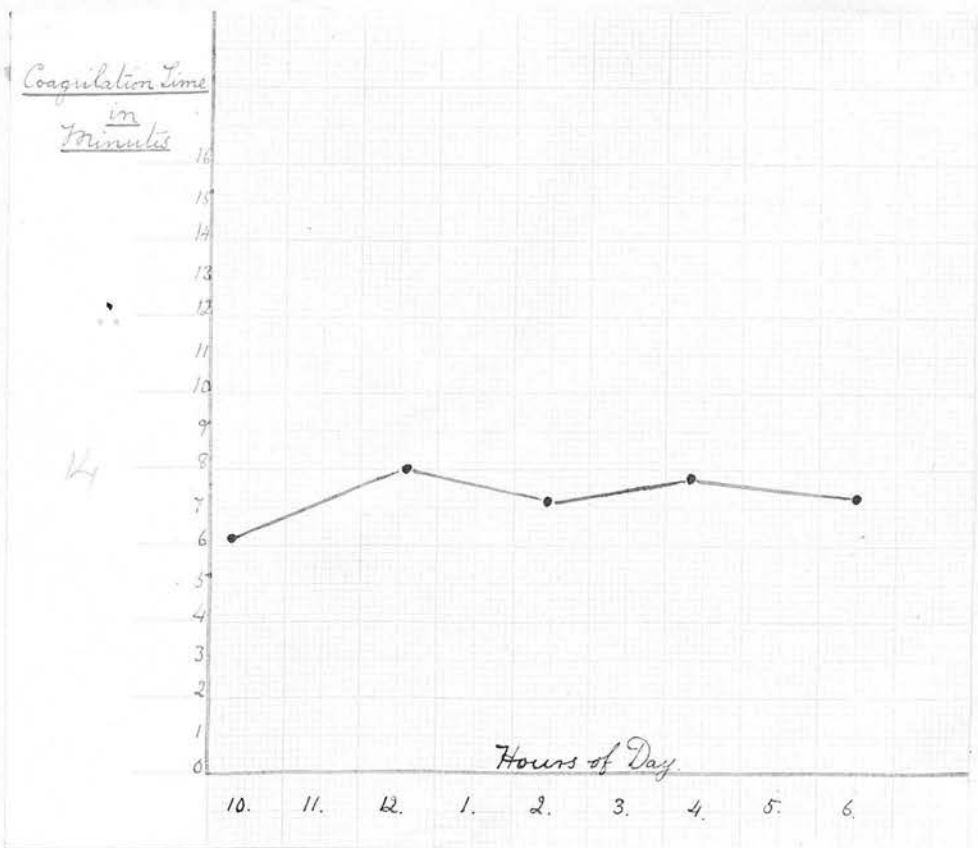
F.C.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
10	59	0	=	7	15
12	51	45	=	7	15
2	56	45	=	7	30
4	18	45	=	7	30



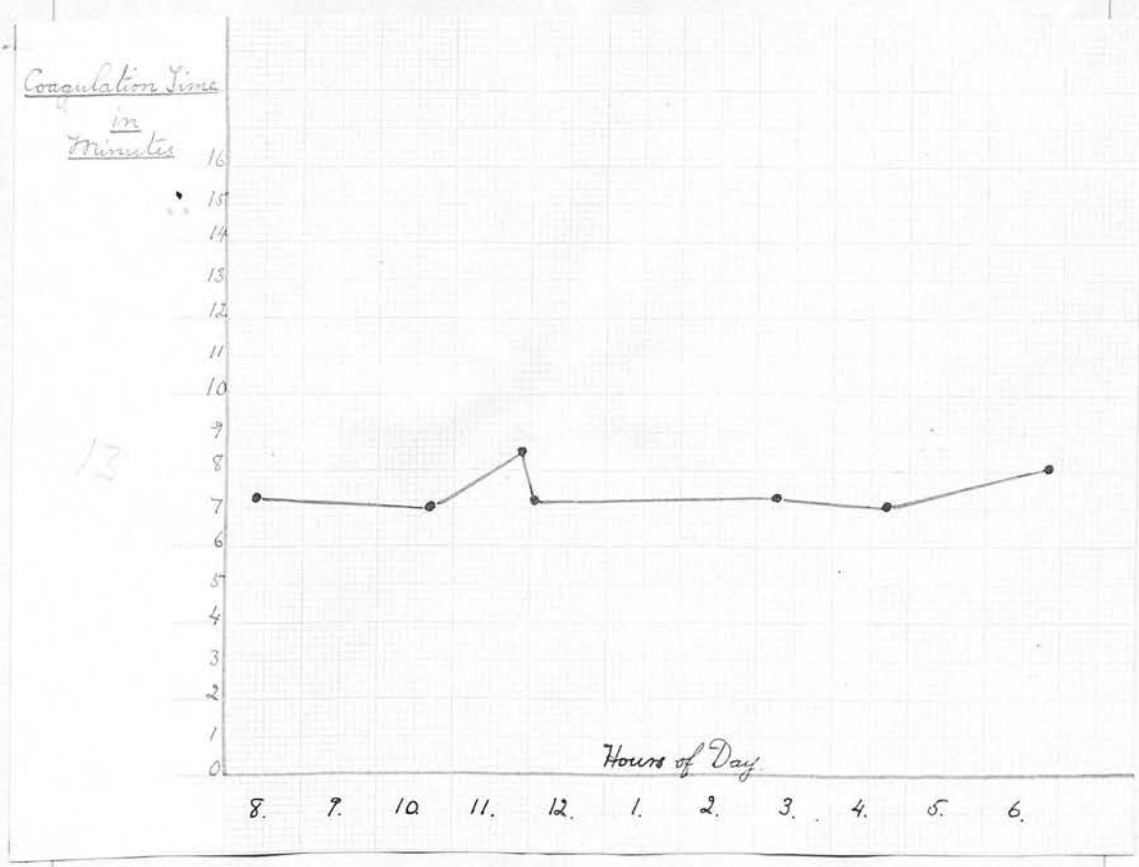
W.R.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
10	17	15	=	6	15
12	37	0	=	8	0
2	24	15	=	7	15
4	20	0	=	7	45
6	29	0	=	7	10



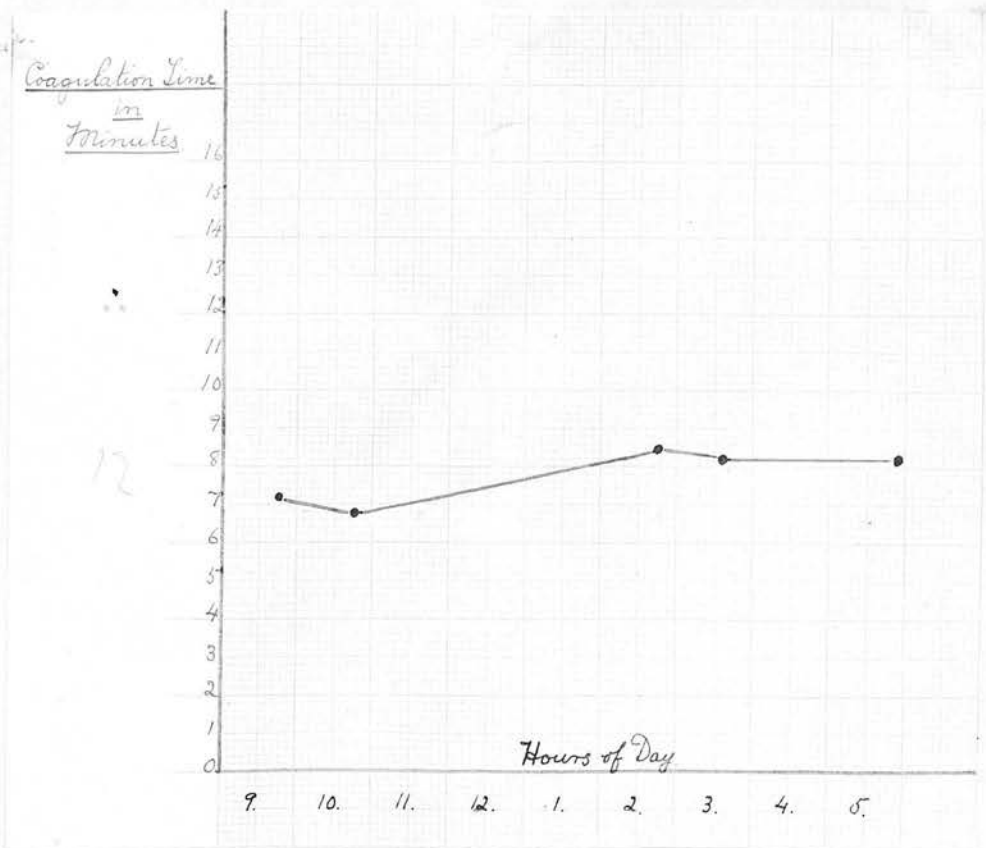
T.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
8	25	33	=	7	20
10	40	30	=	7	0
11	54	0	=	8	30
12	4	30	=	7	15
3	16	15	=	7	20
4	41	40	=	7	5
6	52	45	=	8	5



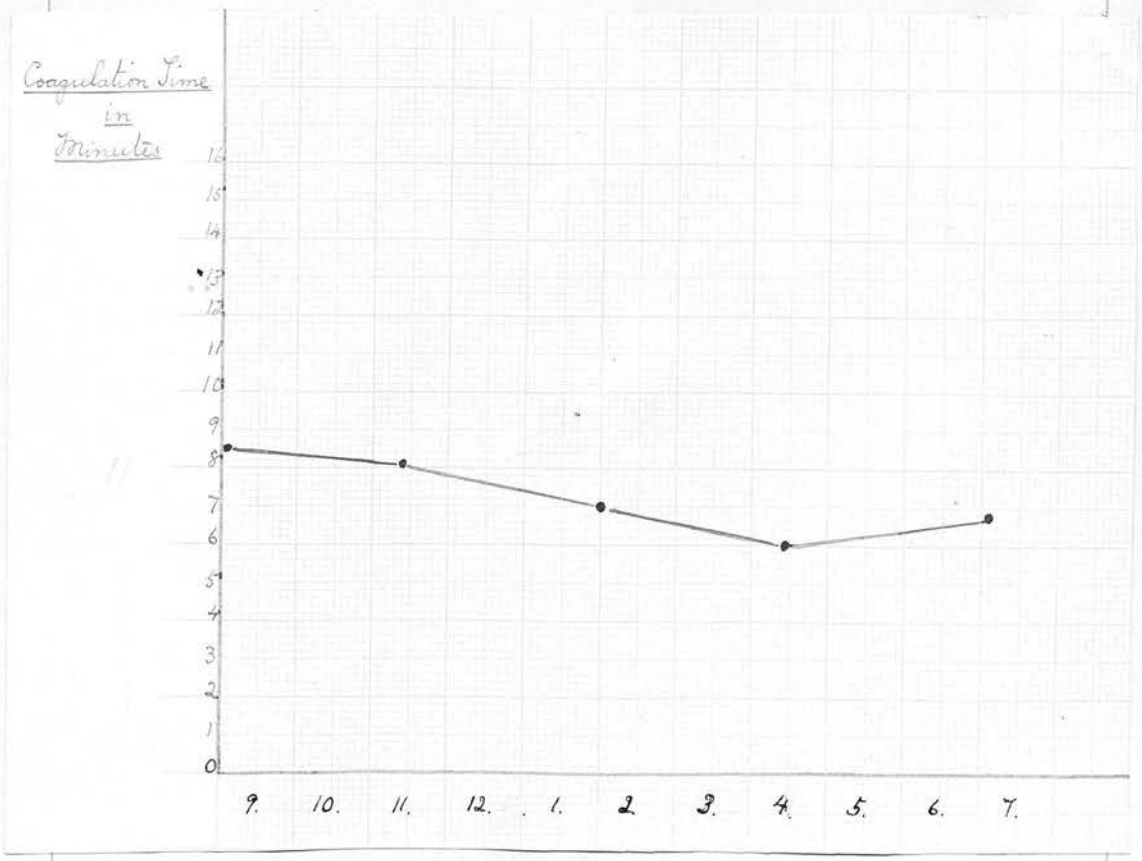
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
9	44	35	=	7	10
10	46	30	=	6	45
2	47	13	=	8	27
3	36	0	=	8	15
5	25	30	=	8	15



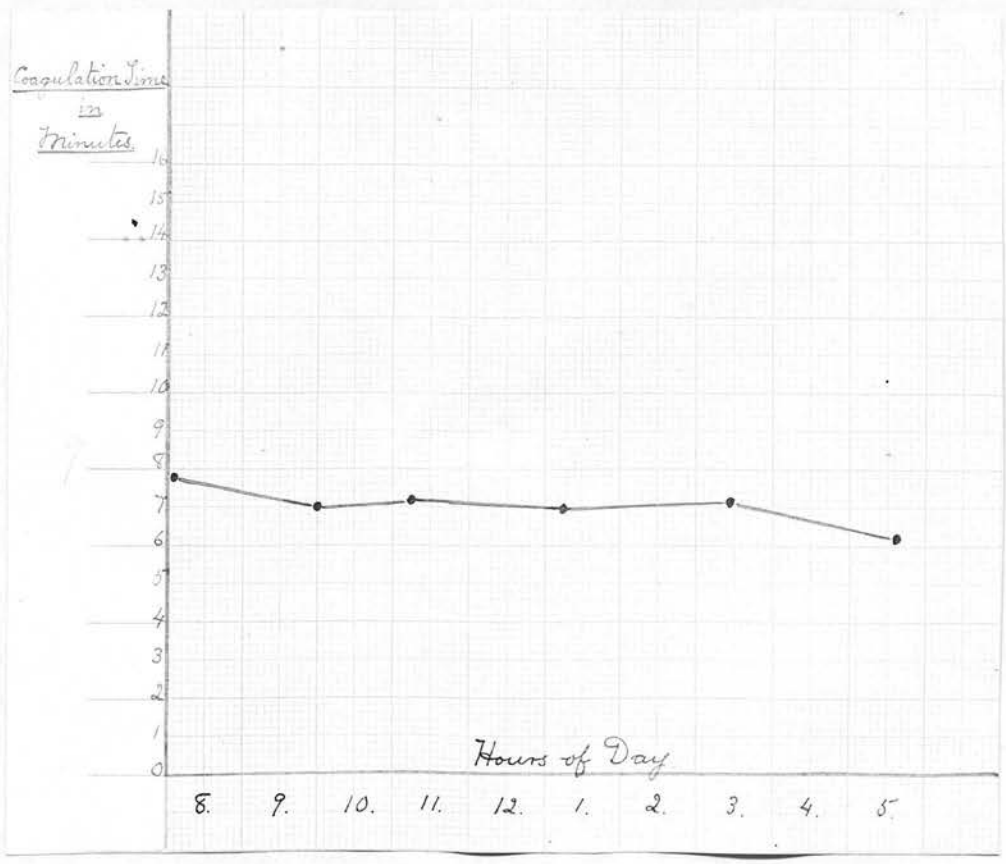
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
9	1	30	=	8	30
11	23	35	=	8	5
1	59	0	=	7	0
4	26	15	=	6	0
7	6	45	=	6	45



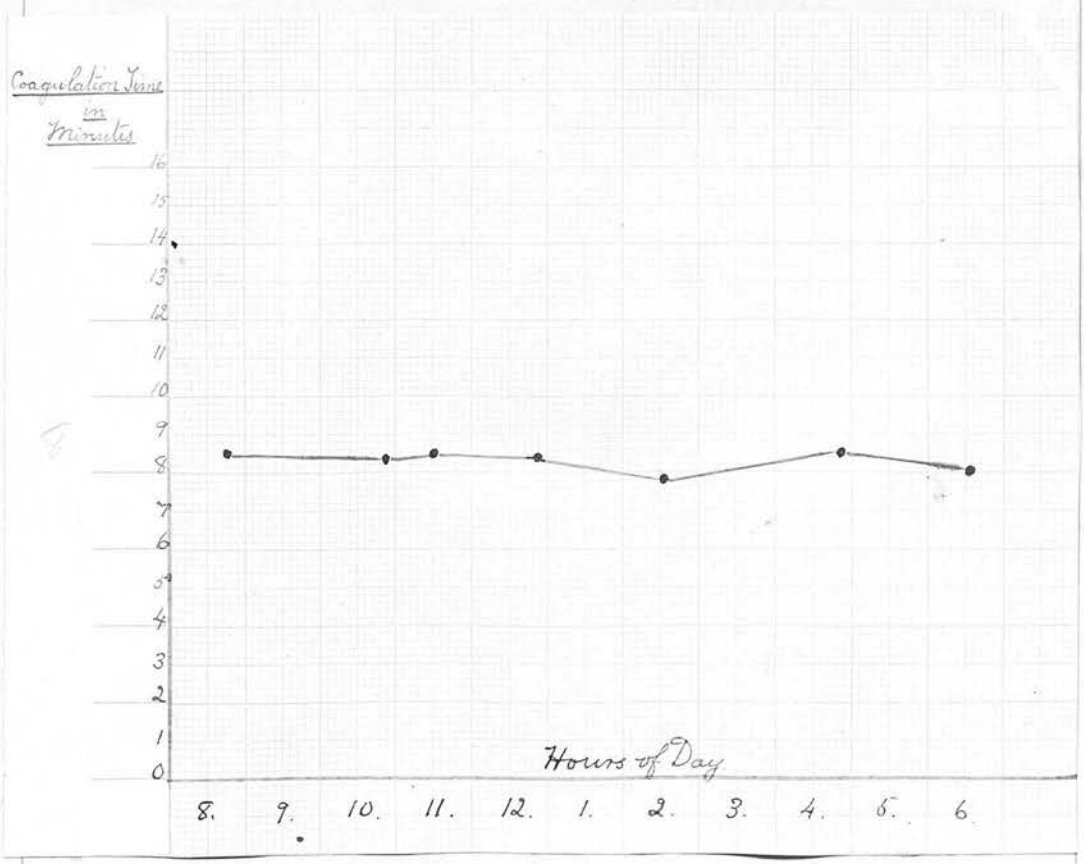
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
8	2	0	=	7	45
10	1	30	=	7	0
11	16	50	=	7	10
1	15	20	=	7	0
3	26	35	=	7	10
5	38	0	=	6	10



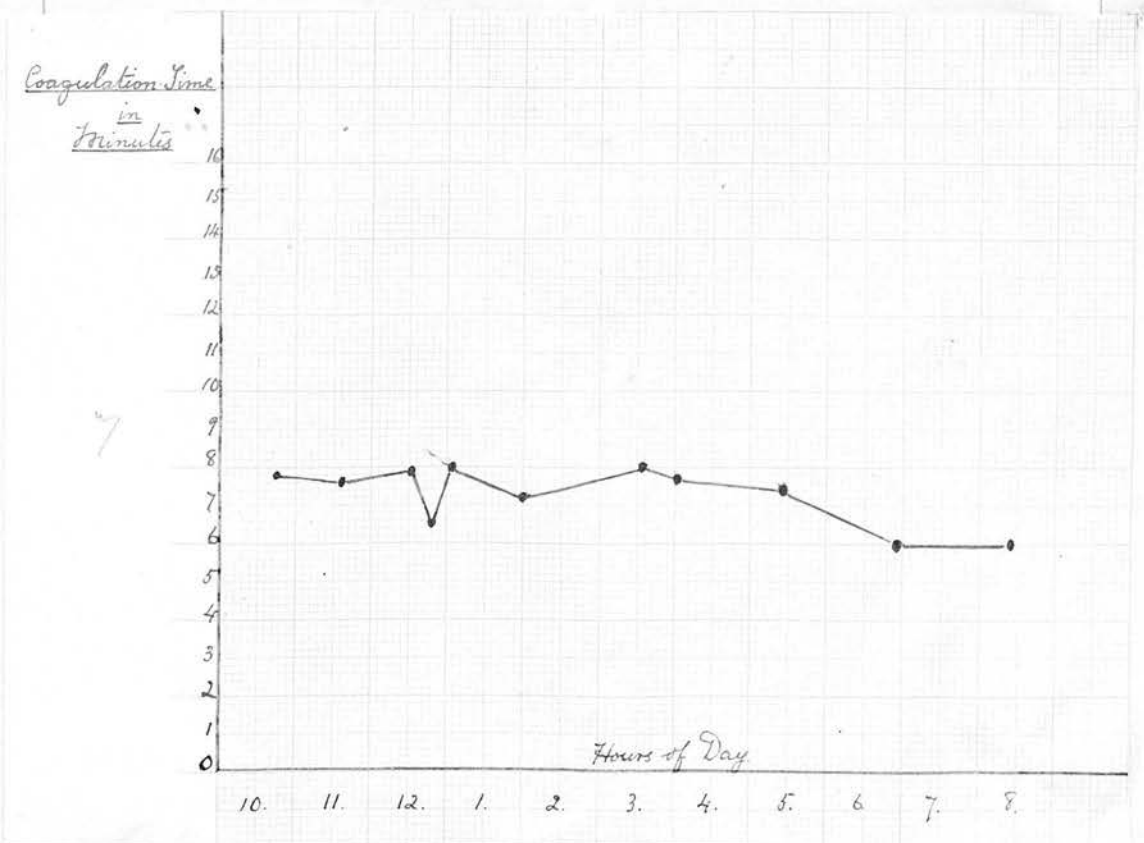
T.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
8	46	45	=	8	30
10	50	35	=	8	25
11	28	0	=	8	30
12	52	50	=	8	25
2	35	30	=	7	50
4	51	0	=	8	30
6	32	10	=	8	5



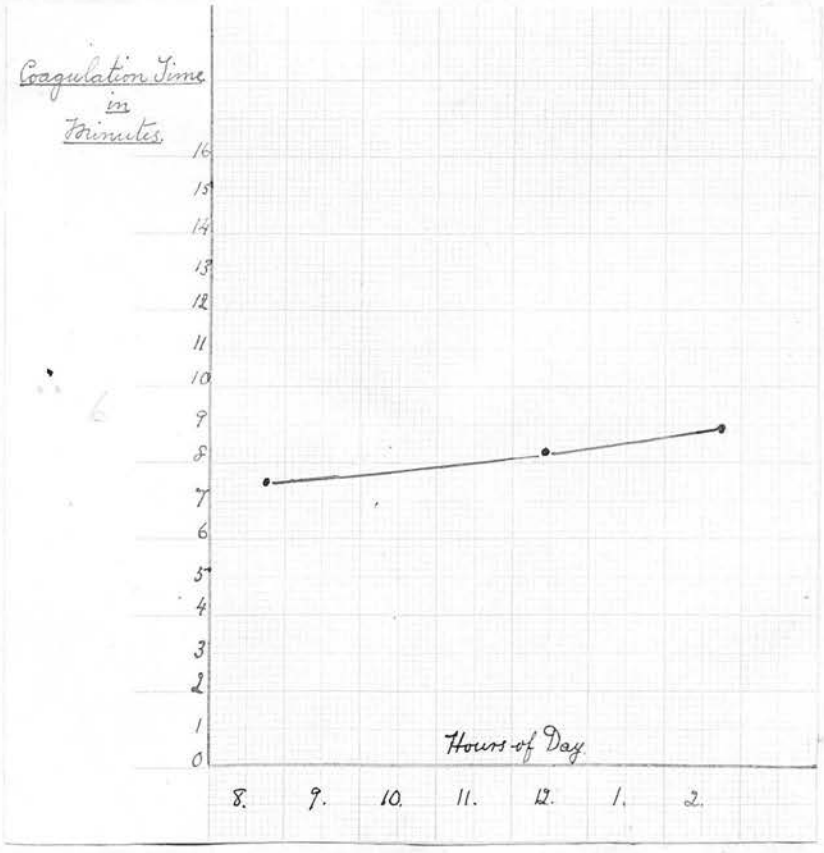
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
10	47	40	=	7	50
11	39	40	=	7	40
12	3	40	=	7	55
12	47	10	=	6	30
1	8	30	=	8	0
1	59	30	=	7	20
3	38	30	=	8	0
4	3	15	=	7	45
5	26	25	=	7	25
6	53	10	=	6	0
8	24	20	=	6	0



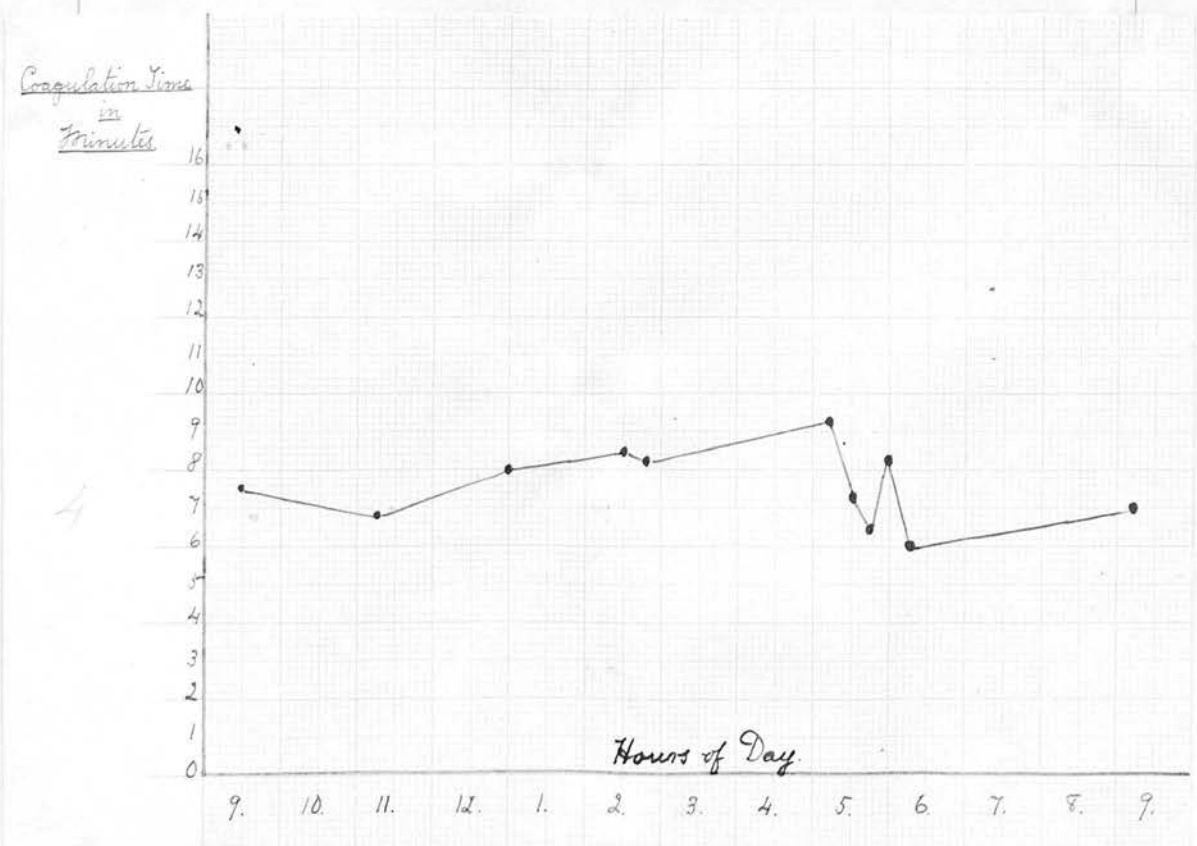
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
8	45	0	=	7	30
12	28	42	=	8	18
2	47	5	=	8	55



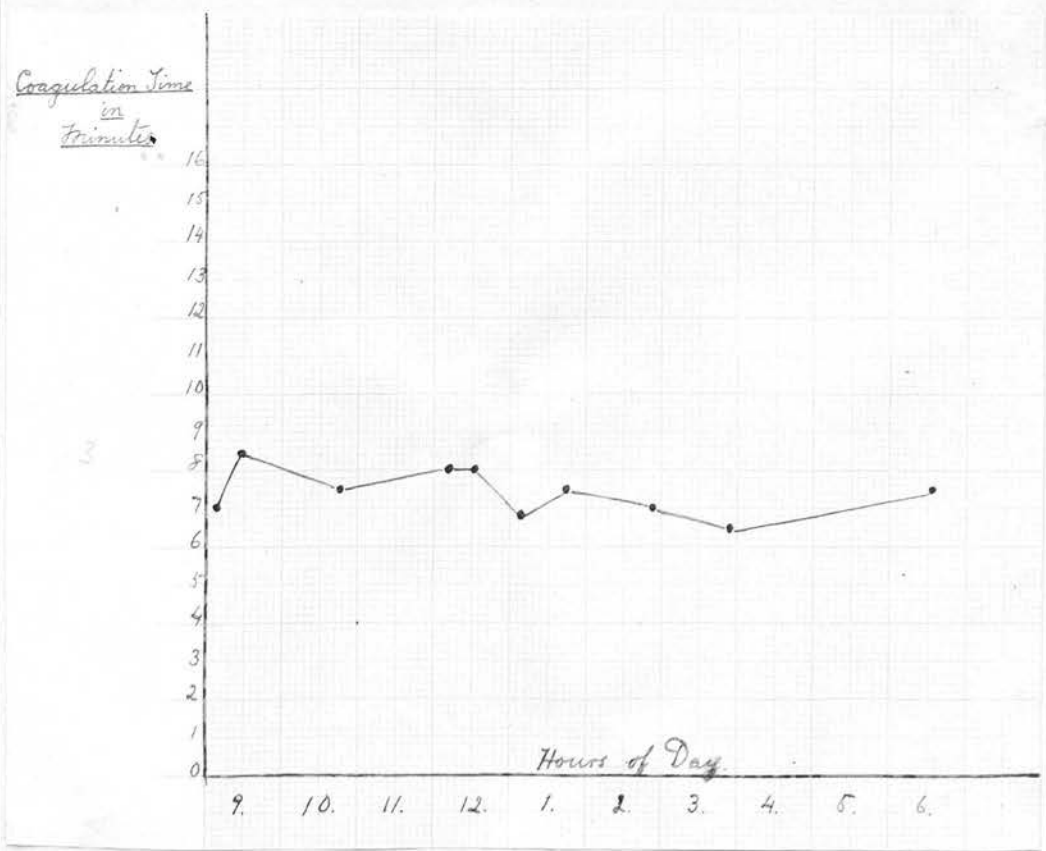
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min	sec.
9	28	10	=	7	35
11	16	35	=	6	45
12	58	55	=	8	0
2	34	15	=	8	30
2	49	35	=	8	10
5	12	15	=	9	15
5	33	28	=	7	17
5	46	35	=	6	25
5	58	15	=	8	15
6	22	0	=	6	0
9	11	30	=	7	0



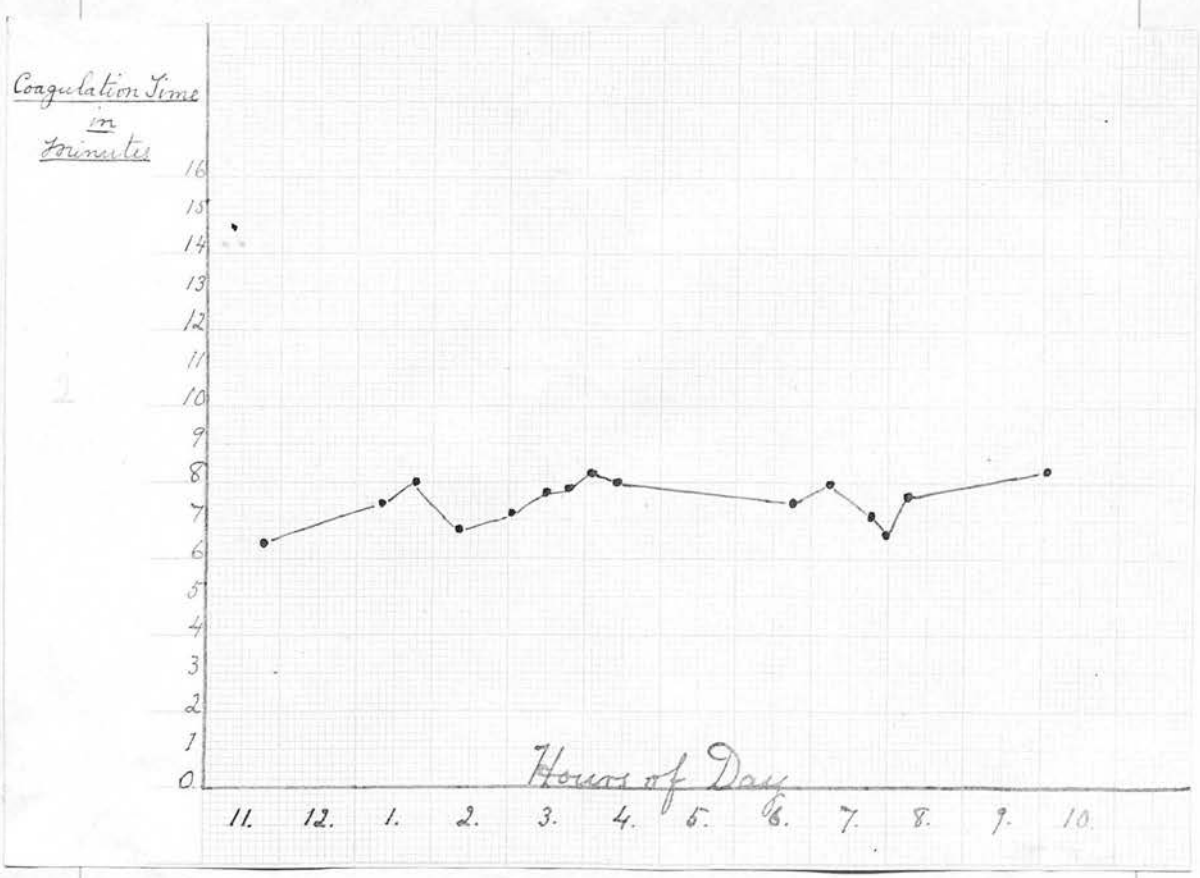
T.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
9	10	0	=	7	0
9	27	30	=	8	30
10	45	45	=	7	25
12	11	40	=	8	0
12	34	40	=	8	0
1	11	0	=	6	40
1	40	15	=	7	30
2	52	45	=	7	0
3	51	45	=	6	35
6	38	15	=	7	30



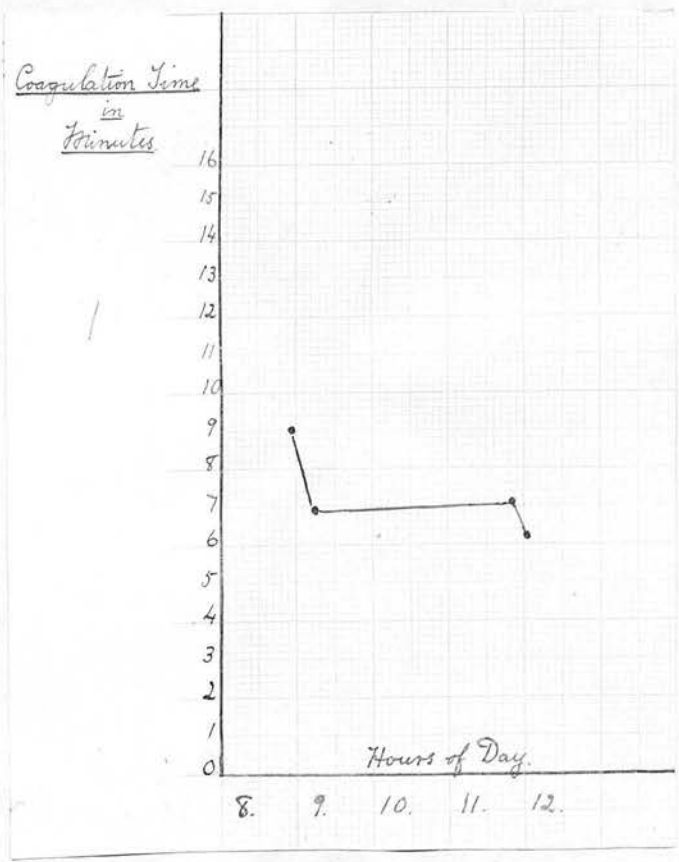
T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
11	43	30	=	6	30
1	19	0	=	7	30
1	41	30	=	8	0
2	17	10	=	6	50
3	2	0	=	7	15
3	26	15	=	7	45
3	43	40	=	7	50
4	6	45	=	8	15
4	23	30	=	8	0
6	42	10	=	7	35
7	14	5	=	7	55
7	44	45	=	7	5
7	58	15	=	6	45
8	13	15	=	7	45
10	6	45	=	8	15



T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
8	56	30	=	9	0
9	18	40	=	6	50
11	50	5	=	7	10
12	3	0	=	6	15



INFLUENCE OF FOOD ON THE COAGULATION TIME.

In the table below coagulation times taken before and after breakfast are given. Food has been described as a cause of variation in the coagulation time by Burker, Coleman and others. The average time before food is 7 min. 47 sec., and the average after food is 7 min. 46 sec., so food appears to have no effect at all. These results may also be taken as showing that exercise, which has also been thought to be a cause of variation, has no effect.

TIME OF DAY. hrs.min.sec.		COAG.TIME min.sec.
8. 45. 0.a.m.	Before food.	7. 30.
12. 28. 42.a.m.	After food.	8. 18.
8. 56. 30 a.m.	Before food.	9. 0.
9. 18. 40.a.m.	After food.	6. 50.
8. 46. 45.a.m.	Before food.	8. 30.
10. 50. 35.a.m.	After food.	8. 25.
8. 2. 0.a.m.	Before food.	7. 45.
10. 1. 30.a.m.	After food.	7. 0.

TIME OF DAY. hrs.min.sec.		COAG.TIME. min.sec.
9. 1. 30.a.m.	Before food. or exercise.	8. 30.
11. 23. 35.a.m.	After food.	8. 5.
8. 25. 33.a.m.	Before food. or exercise.	7. 20.
10. 40. 30.a.m.	After food.	7. 0.
7. 44. 50.a.m.	Before food. or exercise.	7. 25.
8. 53. 45.a.m.	After food.	8. 45.
8. 8. 35.a.m.	Before food. or exercise.	7. 10.
9. 10. 35.a.m.	After food.	8. 10.
7. 44. 50.a.m.	Before food. or exercise.	7. 10.
8. 8. 35.a.m.	After food.	6. 50.
8. 45. 0.a.m.	Before food.	7. 30.
12. 28. 43.a.m.	After food.	8. 18.

THE RELATION BETWEEN THE AMOUNT OF IONISABLE CALCIUM IN THE BLOOD AND ITS COAGULATION TIME.

Calcium is one of the necessary factors in the process of coagulation. By adding Oxalic Acid or Sodium Oxylate to blood, the calcium salts are precipitated and it will no longer coagulate.

Wright[1] and later Horne[2] found that by adding excess of lime salts to blood, coagulation was prevented.

Sabbatini[3] states that a part of the calcium in the blood is in combination with proteid molecules and is in an non-dissociable form. It is only the ionisable calcium which takes part in coagulation. He believes that the Calcium-ion can only join in the formation of fibrin ferment when it is within the limits of a certain minimum and maximum concentration. When the concentration falls too low as after the addition of oxylates, or when it rises too high as when/

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1. Wright. Jour. Path. & Bact. 1893, Vol. I, p. 434

2. Horne. Jour. of Physiol. Camb. & Lond. 1896, Vol XIX. pg. 356.

3. Sabbatini Arch. Ital. de. Biol.

when excess of soluble lime salts are added , coagulation is prevented. He also explains on these lines how Sodium Citrate is able to keep blood from coagulating. For the citrate by uniting with the calcium ions to form calcium citrate, which though soluble is less dissociable than the ordinary calcium salts in the blood, diminishes the ionic concentration below the necessary minimum.

C.G. Martin[1] has gone further and shown that the calcium citrate goes to form a more complex body for which he gives the following formula.

$$\left(C_3H_4(OH) \begin{matrix} COONa \\ COO \\ COO \end{matrix} \begin{matrix} \\ Ca \\ \end{matrix} \right)_{11}$$

Sabbati calculated that three molecules of Sodium citrate were necessary for every dissociable molecule containing calcium in order to produce incoagulability, and Horne found he needed to add to prevent coagulation.

These results were all, of course, obtained by the addition of calcium and citrates to the blood after its withdrawal from the body. It appeared to be worth while to try whether such lesser amounts of Calcium/

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1. Bio-Chemical Journal Vol II, No 4, 1907, from an address to the Lister Institute (unpublished).

Calcium or Citrates as might be absorbed and circulate in the body, might not have some effect on the coagulation time. A method of estimating the amount of ionic calcium in the blood was therefore necessary in order to find whether this taking of calcium and citric acid produced any change in the amount of ionic calcium in the blood. The usual methods of estimating the quantity of calcium in the blood are of no value for this purpose, for they break up the proteid molecules, and the result given is the total calcium, whereas it is the quantity of ionisable calcium which is required.

Fortunately at this time Blair Bell[1] published a method whereby this can be determined.

DESCRIPTION OF METHOD.

One hundred cm. of freshly drawn blood is measured in a special pipette and immediately mixed with 250 cm. of a solution of Oxalic Acid, in a small glass flask which has been carefully cleaned with Aqua Regia and distilled water. After waiting for some time 250 cm. of a solution of Acetic Acid and Glycerine is added. After another interval 100 cm. of this mixture is taken and shaken up with 500 cm. of distilled water in another flask.

A/

1.

A small drop of this last solution is then put on a Thoma-Zeiss blood counting slide, and the number of crystals of calcium oxylate in 250 squares is counted. The crystals are very minute and the counting requires a long time and great care. They are recognized by their showing alternately dark and bright on focussing up and down, and also by their Brownian movement.

If an average of 1 crystal is found in each square, the amount of calcium is equal to 1 part of Calcium Oxide in 6,000 parts of blood. The results are comparative and do not give the actual amount by weight. If the average of crystals per square is 1.5, the calcium index is 1.5, and so on.

This method deals only with the ionisable calcium, for only that part is precipitated by Oxalic Acid.

As this method had just been published and no results have yet been shown, it was necessary to test its accuracy. After using it a good deal and having become thoroughly familiar with the technique, I tried it in the following way.

TESTING of BLAIR BELL'S CALCIMETER.

On eight successive days small quantities of mixed animal serum were obtained from the Slaughter House/

House. In each sample the amount of ionic calcium was determined. The same serum was then diluted to a known extent, with distilled water, or a solution of calcium chloride of known strength was added. From the estimation of the amount of calcium in the undiluted serum, the quantity which ought to be arrived at from examination of the diluted serum was known.

The difference between the actual result, and the result which should have been attained, gave an indication of the extent of experimental error.

I.

1. Serum.

a 2.312 parts in 6000)
b 2.329) = 2.320

2. Serum & equal amount of 1-6000 Calcium Oxide solution

a 1.572 parts in 6000)
b 1.781) = 1.676

1.676 should have been (2.320 ÷ 2) + .5 = 1.635

∴ The error = .041 parts in 6000.

II.

1. Serum = 2.287

2 Serum & equal amount of Distilled Water = 1.162

1.162 should have been 1.143

∴ The Error = .031.

III.

1. Serum = 2.862

2. Serum & equal amount of Distilled Water = 1.608

1.608 should have been 1.431

∴ The Error = .354. (Light so poor that a lower magnification for counting was necessary than in the other estimations.)

IV./

IV.

1. Serum = 2.287

2. Serum & equal amount of Distilled Water = 1.162

1.162 should have been 1.143

∴ The Error = .031.

V.

1. Serum = 2.0195

2. Serum & equal amount of Distilled Water = .8281

.8281 should be 1.009

∴ Error = .181.

VI.

1. Serum = 1.933

2. Serum & equal amount of Distilled Water = .933

.933 should be .666

∴ The Error = .033.

VII.

1. Serum = 1.394

2. Mixture of $^{2}/_{3}$ Serum & $^{1}/_{3}$ Distilled Water = 1.0038

1.0038 should be 1.505

∴ The Error = .502

In/

(In all the other estimations the serum used was never more than 24 hours old. In this case it was a mixture of serum some 72 hours, and some 48 hours old.)

VIII.

1. Serum = 2.367
2. Mixture of $^{2}/_{3}$ Serum and $^{1}/_{3}$ Distilled Water = 1.578

1.578 should be 1.576

∴ The Error = .002.

SUMMARY of RESULTS of TESTING

of

BLAIR BELL'S CALCIMETER.

The Errors

.041	
.031	
.354	(poor light)
.031	
.181	
.033	
.505	(old serum)
.002	
1.178	

∴ The Average Error = 1.178 ÷ 8 = .147.

Dr. Blair Bell pointed out to me that one ought/

ought to subtract from this average error, the error which is brought about by counting as equal, measured volumes of distilled water, or Ca. solution in distilled water, and of serum, which have different densities.

If the two large errors, in both of which circumstances external to the method may have vitiated the result, are excluded, the average error is only .053. In ordinary routine estimations, however, there are similar opportunities of error. The counting of the crystals is difficult and tedious, and the personal equation is a factor which must be taken into account. The crystals vary in size considerably, but the degree of variation in different bloods is remarkably constant, so that this cannot lead to any great error.

An average error of 10% may, on the whole, I think be expected. Although this is no doubt a large error, yet for purely comparative purposes the method still remains of great value, for as I shall shew the temporary variations of the amount of ionic calcium in the blood which may be produced by the administration of Calcium and Citric Acid are so large as to overshadow the experimental error.

THE/

The administration by the mouth of soluble lime salts is followed by an increase in the amount of ionisable calcium in the blood, while citric acid leads to a decrease in the amount.

From the following results it will be seen that the calcium index may be very variable and that the administration of calcium has a very great effect in increasing it. Many of these estimations are the average of two counts.

28th Septr. 1907.
At 12.16 p.m. Calcium Index = 0.31
29th Septr.
At 9.0 a.m. Calcium Index = 0.35
At 6.0 p.m. Sixty grains of Calcium Lactate given
At 7.0 p.m. Calcium Index = 0.52
At 10.45 p.m. Calcium Index = 0.95

30th Septr.
At 8.30 a.m. Calcium Index = 0.68
At 11.0 p.m. Calcium Index = 0.64

1st Octr.
At 8.30 a.m. Calcium Index = 1.21

2nd Octr.
At 11.0 a.m. Calcium Index = 0.64

3rd Octr.
At 4 p.m. Calcium Index = 1.0
At 8 p.m. Thirty grains of Calcium Lactate were given
At 9 p.m. Calcium Index = 1.93

4th/

4th Octr.
At 9.30 a.m. Calcium Index = 1.31
At 8.0 p.m. Calcium Index = 0.92
At 9.20 p.m. Thirty grains of Calcium Lactate were given.
At 10.20 p.m. Calcium Index = 1.50

5th Octr.
At 9 a.m. Calcium Index = 1.29

6th Octr.
At 1.30 p.m. Calcium Index = 1·1

7th Octr.
At 9 a.m. (No food having been taken for 16 hours)
Calcium Index = 0.55
At 9.30 p.m. (Milk diet during day)
Calcium Index = 1.11

9th Octr.
At 8.30 a.m. (No food for 16 hours).
Calcium Index = 1.7

The chart below shows the above variations.

In the next two cases the blood was taken at 5 p.m. every day. The first was a child with acute lobar pneumonia. The second was a child convalescent from acute lobar pneumonia whose heart-action was weak and irregular. It is worthy of remark that in the latter case, coincident with the increase in the amount of calcium in the blood, there was an improvement in the character of the pulse.

1st Acute Lobar Pneumonia.

3rd day of illness — Calcium Index = 1.46

4th day of illness — Calcium Index = 1.04

5th day of illness — Calcium Index = 1.12

6th day of illness — Calcium Index = 0.90

7th day of illness — Calcium Index = 0.82

8th day of illness (Crisis)
At 2 p.m. — Calcium Lactate grs.X was given T. i. d.
At 5 p.m. — Calcium Index = 1.98

9th day of illness — Calcium Index = 1.61

Chart/

Chart.

2nd.Convalescent from acute Lobar pneumonia.

14th Dec.	Calcium Index = 0.55
16th Dec.	Calcium Index = 0.95
17th Dec.	
At 4 p.m.	Calcium Lactate gr. V was given, and was continued thereafter every 4 hours.
At 5 p.m.	Calcium Index = 1.19
18th Dec.	Calcium Index = 1.03
19th Dec.	Calcium Index = 1.50

The/

The following experiments show the results of the administration of calcium in healthy people.

T.A.

At 10.30 a.m.	Calcium Index = 0.93
At 10.35 a.m	Forty grains of Calcium Lactate were taken.
At 11.48 a.m.	Calcium Index = 1.28

L.H.

At 12.30 p.m.	Calcium Index = 0.49
At 1.15 p.m.	Forty grains of Calcium Lactate were taken.
At 3.20 p.m.	Calcium Index = 0.67

F.C.A.

At 11.52 a.m.	Calcium Index = 0.91
At 11.55 a.m.	Forty grains of Calcium Lactate were taken.
At 12.50 p.m.	Calcium Index = 1.14

The next two experiments show a diminution in the amount of calcium after citric acid.

W.R.A.

At 11 a.m.	Calcium Index = 0.92
At 11.10 a.m.	Sixty grains of Citric Acid were taken.
At 12.45 p.m.	Calcium Index = 0.67

F.C.A.

At 11 a.m.	Calcium Index = 0.70
At 11.10 a.m.	Sixty grains of Citric Acid were taken.
At 1.25 p.m.	Calcium Index = 0.60

2. THE ADMINISTRATION OF CALCIUM AND CITRIC ACID, ALTHOUGH THEY CHANGE THE AMOUNT OF IONISABLE CALCIUM IN THE BLOOD, DO NOT CAUSE ANY ALTERATION IN THE COAGULATION TIME.

(1)WRIGHT'S RESULTS.

Wright states that in healthy people calcium (1) produces a diminution and citric acid (2) a lengthening of the coagulation time. He has aroused a great deal of interest in the subject by his practical application of this in the treatment of haemorrhage, and also by his assertion that a number of pathological conditions such as chilblains, urticaria, physiological albuminuria. certain forms of diarrhoea and effusions into serous cavities are associated with a diminished coagulability of the blood and that these conditions may be cured or ameliorated by the administration of calcium.

It is only his results in healthy people however/

(1) Brit. Med. Journal 1893 ii. 225.
(2) Lancet Dec. 6th 1902.

however, that I am in a position to discuss.

Alone or in association with others, he has published nine papers on the effect of calcium and citric acid. On going over them I find that he gives thirteen experiments on healthy people in which he gives the coagulation time before and after taking Calcium and two similar experiments on the effect of Citric Acid.

In all of them he obtains a diminution of the time after Calcium Chloride and Calcium Lactate and an increase after Citric Acid. The following are examples of his results.

CALCIUM.

B. M. J. 1893 ii. 225.

L.R.

Coagulation Time = 5½ min.

Calcium Chloride gr. 30 taken.

Coagulation Time 24 hours later = 1¾ min.

Lancet 1905 ii. 1098.

A/

A HEALTHY MAN.

6th Feb.

2 pm. Coagulation Time = 1 min. 50 sec.

2·20 pm. Sixty grains of Calcium Lactate taken

2·30 pm. Coagulation Time = 1 min. 50 sec.

2·40 pm. Coagulation Time = 1 min. 20 sec.

3·50 pm. Coagulation Time = 25 sec.

7th Feb.

Coagulation Time = 1 min. 50 sec.

10th Feb.

Coagulation Time = 45 sec.

CITRIC ACID.

B. M. J. 1894 ii. 57.

1·45 pm. Coagulation Time = 5 min.

4·15 pm. Coagulation Time = 3 min.10 sec.

4·30 pm. Seventy-five grains of Citric Acid taken.

6·40 pm. Coagulation Time = 7 min.15 sec.

9·45 pm. Coagulation Time = 7 min. 0 sec.

11·45 pm. Coagulation Time = 3 min.40 sec.

These/

These results are few in number and when examined in detail, not very convincing. Thus when he finds very little diminution after calcium he assumes that it has not been absorbed, when he finds that the continuous administration of calcium does not keep the coagulation time short, he assumes that there is an excess of calcium salts in the blood, but he does not prove these points by estimating the quantity of calcium.

And yet he has devised a method of determining the calcium content. He measures it in terms of the minimum strength of ammonium oxylate solution necessary to keep the blood from coagulating.(1)

In using this method he was not able to show any connection between the amount of calcium and the coagulation time, but if that is so how does he explain the action of calcium and citric acid?

The enormous reductions in the time which he shows with his latest method (e.g. 30 sec. before, to 35 sec. after Calcium) can, I think with certainty be ascribed to the defect in his end-point which has been previously discussed.

If/

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(1) Lancet 1902 ii. 1531.

If the blood be simply aspirated into the tube and blown out immediately, a fibrin thread can sometimes be demonstrated at once by touching the blood with the end of the fine glass tube and withdrawing it a little. This is not ferment-produced but mechanically produced fibrin.

In using Wright's method I did not find any constant change after Calcium or Citric Acid.

T.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
9	11	0	am.	3	0
9	20	30		2	30
9	35	0	Sixty grains of Calcium Lactate taken.		
11	18	25		2	30
11	28	40		3	15

T.A.

TIME OF DAY hrs.	min.	sec.		COAGULATION TIME. min.	sec.
7	0	0		3	5
7	20	0	Calcium Lactate grs. 60 taken.		
8	22	40		3	15

T./

T.A.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
9	30	0		2	45
9	45	0	Calcium Lactate grs. 60 taken.		
10	43	20		3	20

F.B.

TIME OF DAY. hrs.	min.	sec.		COAGULATION TIME. min.	sec.
5	41	0		2	45
5	40	0	Calcium Lactate grs. 60 taken.		
6	50	30		2	45

2. RESULTS WITH MY METHOD SHEWING THAT CALCIUM AND CITRIC ACID DO NOT ALTER THE COAGULATION TIME.

The Calcium was in some cases given in the form of calcium lactate, completely dissolved in water, in others it was partially dissolved and the remainder was held in suspension by tragacanth powder. The usual dose was 60 grains, but the effect of doses from $7\frac{1}{2}$ to 120 grains were also tried.

The coagulation time was taken before the administration of the calcium, or citric acid. It was taken again at varying intervals of time after the dose. Wright says that the greatest effect of Calcium is found in three quarters of an hour, and in many cases this was the time chosen, but in a number of others the interval was from one to eight hours long.

On looking over these results it will be seen that no constant alteration in the time follows on the taking of either Calcium or Citric Acid. The changes which do occur are sometimes one way, and sometimes another. They are the variations which have been noted as due to experimental errors.

A/

A. Cases showing that, although the Calcium or Citric Acid had altered the amount of ionisable calcium, there was no change in the coagulation time beyond the limits of experimental error.

T.A.

At 10. 10. a.m. The Coagulation Time = 7 min. 15 sec.

At 10.30 a.m. The Calcium Index was 0.93

At 10.30 a.m. forty grains of Calcium Lactate were taken

At 11.37 a.m. The Coagulation Time = 7 min. 45 sec.

At 11.48 a.m. The Calcium Index was 1.28

F.C.A.

At 10.50 a.m. The Coagulation Time = 7 min. 20 sec.

At 11.52 a.m. the Calcium Index was 0.91

At 11.55 a.m. forty grains of Calcium lactate were taken

At 12.50 p.m. the Calcium Index was 1.14

At 1.1 p.m. The Coagulation Time = 7 min. 10 sec.

At 3.39 p.m. The Coagulation Time = 7 min. 10 sec.

N.H.

At 11.44 a.m. The Coagulation Time = 8 min. 25 sec.

At 12.30 p.m. the Calcium Index was 0.49

At 1.15 p.m. forty grains of Calcium Lactate were taken

At/

At 1.34 p.m. The Coagulation Time = 8 min. 20 sec.

At 2.47 p.m. The Coagulation time = 8 min. 5 sec.

At 3.20 p.m. the Calcium Index was 0.67

At 4.32 p.m. The Coagulation time = 7 min. 47 sec.

F.C.A.

At 10.23 a.m. the Coagulation Time = 9 min. 25 sec.

At 11 a.m. the Calcium Index was 0.7

At 11.10 a.m. sixty grains of Citric Acid were taken

At 1.25 p.m. The Calcium Index was 0.60

At 1.45 p.m. The Coagulation Time = 8 min. 0 sec.

W.R.A.

At 11.6 a.m. The Coagulation Time = 7 min. 45 sec.

At 11 a.m. the Calcium Index was 0.92

At 11.10 a.m. sixty grains of Citric Acid were taken

At 12.45 p.m. the Calcium Index was 0.67

At 1.14 p.m. the Coagulation Time = 6 min. 10 sec.

At 3.56 p.m. the Coagulation Time = 7 min. 0 sec.

At 6.35 p.m. The Coagulation Time = 6 min. 35 sec.

B. Cases showing that the administration of Calcium by the mouth has no effect on the Coagulation Time.

P.M.

At 11.12 a.m. The Coagulation Time = 6 min. 45 sec.

11.15 a.m. one hundred and twenty grains of Calcium Lactate were taken

12.8 p.m. The Coagulation Time = 7 min. 30 sec.

F.B.

At 5.24 p.m. The Coagulation Time = 7 min. 25 sec.

5.40 p.m. sixty grains of Calcium Lactate were taken

6.26 p.m. The Coagulation Time = 7 min. 25 sec.

T.A.

At 9.20 a.m. the Coagulation Time = 7 min. 5 sec.

9.30 a.m. sixty grains of Calcium Lactate were taken

10.50 a.m. the Coagulation time = 7 min. 30 sec.

M.M.

At 10.42 a.m. the Coagulation Time = 8 min. 0 sec.

At 11 a.m. sixty grains of Calcium Lactate were taken

At 12.24 p.m. The Coagulation Time = 9 min. 0 sec.

T/

T.A.

At 10.15 a.m. The Coagulation Time = 7 min. 0 sec.

11 a.m. sixty grains of Calcium Lactate were taken

12 noon the Coagulation Time = 8 min. 20 sec.

1.59 p.m. The Coagulation Time = 8 min. 20 sec.

T.A.

At 7.47 a.m. The Coagulation Time = 6 min. 40 sec.

8.18 a.m. The Coagulation Time = 7 min. 50 sec.

8.52 a.m. The Coagulation Time = 7 min. 55 sec.

10.37 a.m. The Coagulation Time = 8 min. 23 sec.

10.45 a.m. sixty grains of Calcium Lactate were taken

11.59 a.m. **The Coagulation Time** = 7 min. 35 sec.

1.15 p.m. The Coagulation Time = 6 min. 45 sec.

2.10 p.m. The Coagulation Time = 8 min. 30 sec.

A.W.

At 2.45 p.m. sixty grains of Calcium Lactate were taken

3.10 p.m. The Coagulation Time = 8 min. 10 sec.

4.7.p.m. The Coagulation Time = 9 min. 55 sec.

G/

G.G.

At 10.34 p.m. The Coagulation Time = 8 min. 30 sec.

10. 40 p.m. sixty grains of calcium lactate were taken

11.32 p.m. The Coagulation Time = 10 min. 30 sec.

11.50 p.m. The Coagulation Time = 8 min. 15 sec.

E.R.

At 4.48 p.m. The Coagulation Time = 8 min. 20 sec.

5.13 p.m. sixty grains of Calcium Lactate were taken

5.54 p.m. The Coagulation Time = 9 min. 30 sec.

T.A.

At 8.49 a.m. The Coagulation Time = 6 min. 40 sec.

9.35 a.m. sixty grains of Calcium Lactate were taken

5.1. p.m. The Coagulation Time = 7 min. 25 sec.

At 9.0. a.m. The Coagulation Time = 8 min. 20 sec.

10.10. a.m. sixty grains of Calcium Lactate were taken

5.13 p.m. The Coagulation Time = 8 min. 45 sec.

A/

A.G.

At 4.30 p.m. The Coagulation Time = 8 min. 0 sec.

4.35 p.m. sixty grains of Calcium Lactate were taken

5.58 p.m. The Coagulation Time = 6 min. 50 sec.

T.A.

At 1.59 p.m. The Coagulation Time = 7 min. 20 sec.

3.15 p.m. sixty grains of Calcium Lactate were taken

3.35 p.m. The Coagulation Time = 8 min. 0 sec.

4.3 p.m. The Coagulation Time = 7 min. 45 sec.

5.26 p.m. The Coagulation Time = 7 min. 25 sec.

6.53 p.m. The Coagulation Time = 6 min. 0 sec.

8.24 p.m. The Coagulation Time = 6 min. 0 sec.

F.C.A.

At 11.8 a.m. The Coagulation Time = 8 min. 55 sec.

11.10 a.m. forty grains of Calcium Lactate were taken

11.57 a.m. The Coagulation Time = 8 min. 23 sec.

1.52 p.m. The Coagulation Time = 7 min. 55 sec.

4.18 p.m. The Coagulation Time = 7 min. 35 sec.

T/

T.A.

At 5.42 p.m. The Coagulation Time = 9 min. 0 sec.

6.40 p.m. The Coagulation Time = 9 min. 13 sec.

8.30 p.m. forty grains of Calcium Lactate were taken

10.0 p.m. The Coagulation Time = 10 min. 30 sec.

F.C.A.

At 5.59 p.m. The Coagulation Time = 9 min. 0 sec.

6.56 p.m. The Coagulation Time = 10 min. 30 sec.

8.30 p.m. forty grains of Calcium Lactate were taken

10.32 p.m. The Coagulation Time = 10. min. 0 sec.

L.F.

At 10.7 p.m. The Coagulation Time = 9 min. 3 sec.

10.10 p.m. thirty grains of Calcium Lactate were taken

10.56 p.m. The Coagulation Time = 8 min. 45 sec.

T.A.

At 10.47 a.m. The Coagulation Time = 7 min. 50 sec.

11.39 a.m. The Coagulation Time = 7 min. 40 sec.

12 noon twenty grains of Calcium Lactate were taken

12.3 p.m. The Coagulation Time = 7 min. 55 sec.

12.47 p.m. The Coagulation Time = 6 min. 30 sec.

1.8 p.m. The Coagulation Time = 8 min. 0 sec.

P/

P.C.

At 6.20 p.m. fifteen grains of Calcium Lactate were taken

6.30 p.m. The Coagulation Time = 8 min. 35 sec.

7.29 p.m. The Coagulation Time = 8 min. 15 sec.

J.C.

At 7.15 p.m. The Coagulation Time = 8 min. 50 sec.

7.20 p.m. fifteen grains of Calcium Lactate were taken

8.44 p.m. The Coagulation time = 9 min. 25 sec.

D.C.

At 7.58 p.m. The Coagulation Time = 6 min.

8.0 p.m. seven and a half grains of Calcium Lactate were taken

8.45 p.m. The Coagulation time = 8 min. 20 sec.

J.C.

At 8.13 p.m. The Coagulation Time = 7 min. 30 sec.

8.15 p.m. seven and a half grains of Calcium Lactate were taken

10.7 p.m. The Coagulation Time = 8 min. 20 sec.

C. Cases showing that the administration of Citric Acid by the mouth has no effect on the Coagulation Time.

P.M.

At 4.10 p.m. The Coagulation Time = 7 min. 10 sec.

4.15 p.m. one hundred and twenty grains of Citric Acid were taken

5.34 p.m. The Coagulation Time = 7 min. 15 sec.

D.W.P.

At 11.3 a.m. The Coagulation Time = 8 min. 45 sec.

11.5 a.m. sixty grains of Citric Acid were taken

12.9 p.m. The Coagulation Time = 9 min. 15 sec.

C.L.

At 8.20 a.m. The Coagulation Time = 7 min. 30 sec.

8.45 a.m. sixty grains of Citric Acid were taken

9.6 a.m. The Coagulation Time = 7 min. 30 sec.

F.B.

At 8.43 a.m. The Coagulation Time = 7 min. 0 sec.

8.45 a.m. Sixty grains of Citric Acid were taken

9.33 a.m. The Coagulation Time = 6 min. 50 sec.

M/

M.T.

9.5. a.m. The Coagulation Time = 7 min. 30 sec.

9.52 a.m. sixty grains of Citric Acid were taken

12.31 p.m. The Coagulation Time = 7 min. 45 sec.

R.

At 2.53 p.m. The Coagulation Time = 7 min. 0 sec.

3 p.m. sixty grains of Citric Acid were taken

3.49 p.m. The Coagulation Time = 6 min. 50 sec.

T.A.

At 9.8 a.m. The Coagulation Time = 8 min. 30 sec.

9.15 a.m. sixty grains of Citric Acid were taken

10.46 a.m. The Coagulation Time = 8 min. 30 sec.

E.W.

At 10.57 a.m. The Coagulation Time = 9 min. 35 sec.

11.0 a.m. sixty grains of Citric Acid were taken

11.56 a.m. The Coagulation Time = 7 min. 30 sec.

P.G.

At 11.32 a.m. The Coagulation Time = 8 min. 50 sec.

11.35 a.m. one hundred & twenty grains of Citric Acid were taken

12.25 p.m. The Coagulation Time = 9 min. 20 sec.

P/

P.C.

At 7.35 p.m. The Coagulation Time = 8 min. 25 sec.

7.40 p.m. sixty grains of Citric Acid were taken

9.41 p.m. The Coagulation Time = 9 min. 30 sec.

D.C.

At 6.52 p.m. The Coagulation Time = 8 min. 0 sec.

7.4 p.m. The Coagulation Time = 9 min. 0 sec.

7.12 p.m. sixty grains of Citric Acid were taken

8.0 p.m. The Coagulation Time = 8 min. 0 sec.

E.R.

At 10.8 a.m. The Coagulation Time = 7 min. 30 sec.

10.10 a.m. sixty grains of Citric Acid were taken

1.13 p.m. The Coagulation Time = 8 min. 55 sec.

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